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(54) Title: USE OF HEREGULIN AS AN EPITHELIAL CELL GROWTH FACTOR

(57) **Abstract**

Ligands which bind to the HER2, HER3 and/or HER4 receptors are useful as normal epithelial cell growth factors.

USE OF HEREGULIN AS AN EPITHELIAL CELL GROWTH FACTOR

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to the use of HER2, HER3 and/or HER4 ligands, in particular heregulin polypeptides, as epithelial cell growth factors.

Description of Background and Related Art

The HER (ErbB) family belongs to the subclass I receptor tyrosine kinase superfamily and consists of three distinct receptors, HER2, HER3, and HER4. A ligand for this ErbB family is the protein heregulin (HRG), a multidomain containing protein with at least 15 distinct isoforms.

Transduction of signals that regulate cell growth and differentiation is regulated in part by phosphorylation of various cellular proteins. Protein tyrosine kinases are enzymes that catalyze this process. Receptor protein tyrosine kinases are believed to direct cellular growth via ligand-stimulated tyrosine phosphorylation of intracellular substrates. Growth factor receptor protein tyrosine kinases of the class I subfamily include the 170 kDa epidermal growth factor receptor (EGFR) encoded by the *erb*B1 gene. *erb*B1 has been causally implicated in human malignancy. In particular, increased expression of this gene has been observed in more aggressive carcinomas of the breast, bladder, lung and stomach.

The second member of the class I subfamily, $p185^{neu}$ was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. The *neu* gene (also called *erb*B2 and HER2) encodes a 185 kDa receptor protein tyrosine kinase. Amplification and/or overexpression of the human HER2 gene correlates with a poor prognosis in breast and ovarian cancers (Slamon *et al.*, *Science* 235:177-182 (1987); and Slamon *et al.*, *Science* 244:707-712 (1989)). Overexpression of HER2 has been correlated with other carcinomas including carcinomas of the stomach, endometrium, salivary gland, lung, kidney, colon and bladder. Accordingly, Slamon *et al.* in US Pat No. 4,968,603 describe and claim various diagnostic assays for determining HER2 gene amplification or expression in tumor cells. Slamon *et al.* discovered that the presence of multiple gene copies of HER2 oncogene in tumor cells indicates that the disease is more likely to spread beyond the primary tumor site, and that the disease may therefore require more aggressive treatment than might otherwise be indicated by other diagnostic factors. Slamon *et al.* conclude that the HER2 gene amplification test, together with the determination of lymph node status, provides greatly improved prognostic utility.

A further related gene, called *erb*B3 or HER3, has also been described. See US Pat. No. 5,183,884; Kraus *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9193-9197 (1989); EP Pat Appln No 444,961A1; and Kraus *et al.*, *Proc. Natl. Acad. Sci. USA* 90:2900-2904 (1993). Kraus *et al.* (1989) discovered that markedly elevated levels of *erb*B3 mRNA were present in certain human mammary tumor cell lines indicating that *erb*B3, like *erb*B1 and *erb*B2, may play a role in human malignancies. Also, Kraus *et al.* (1993) showed that EGF-dependent activation of the ErbB3 catalytic domain of a chimeric EGFR/ErbB3 receptor resulted in a proliferative response in transfected NIH-3T3 cells. This is now believed to be the result of endogenous ErbB1 or ErbB2 in NIH-3T3. Furthermore, these researchers demonstrated that some human mammary tumor cell lines display a significant elevation of steady-state ErbB3 tyrosine phosphorylation further indicating that this receptor may play a role in human malignancies. The role of *erb*B3 in cancer has been explored by others. It has been found to be overexpressed in breast (Lemoine *et al.*, *Br. J. Cancer* 66:1116-1121 (1992)), gastrointestinal (Poller *et al.*, *J. Pathol.* 168:275-280 (1992), Rajkumer *et al.*, *J. Pathol.* 170:271-278 (1993), and Sanidas *et al.*, *Int. J. Cancer* 54:935-940 (1993)), and pancreatic cancers (Lemoine *et al.*, *J. Pathol.* 168:269-273 (1992), and Friess *et al.*, *Clinical Cancer Research* 1:1413-1420 (1995)).

The class I subfamily of growth factor receptor protein tyrosine kinases has been further extended to

do not have the complete glycosylated spacer region between the Ig-like domain and EGF-like domain. Only one of the GGFs, GGFII, possessed a N-terminal signal peptide. See also WO 92/18627; WO 94/00140; WO 94/04560; WO 94/26298; and WO 95/32724 which refer to GGFs and uses thereof.

Ho *et al.* in *J. Biol. Chem.* 270(4):14523-14532 (1995) describe another member of the heregulin family called sensory and motor neuron-derived factor (SMDF). This protein has an EGF-like domain characteristic of all other heregulin polypeptides but a distinct N-terminal domain. The major structural difference between SMDF and the other heregulin polypeptides is the lack in SMDF of the Ig-like domain and the "glyco" spacer characteristic of all the other heregulin polypeptides. Another feature of SMDF is the presence of two stretches of hydrophobic amino acids near the N-terminus.

While the heregulin polypeptides were first identified based on their ability to activate the HER2 receptor (see Holmes *et al.*, *supra*), it was discovered that certain ovarian cells expressing *neu* and *neu*-transfected fibroblasts did not bind or crosslink to NDF, nor did they respond to NDF to undergo tyrosine phosphorylation (Peles *et al.*, *EMBO J.* 12:961-971 (1993)). This indicated another cellular component was necessary for conferring full heregulin responsiveness. Carraway *et al.* subsequently demonstrated that ^{125}I-rHRG$\beta1_{177-244}$ bound to NIH-3T3 fibroblasts stably transfected with bovine *erb*B3 but not to non-transfected parental cells. Accordingly, they conclude that ErbB3 is a receptor for HRG and mediates phosphorylation of intrinsic tyrosine residues as well as phosphorylation of ErbB2 receptor in cells which express both receptors. Carraway *et al.*, *J. Biol. Chem.* 269(19):14303-14306 (1994). Sliwkowski *et al.*, *J. Biol. Chem.* 269(20):14661-14665 (1994) found that cells transfected with HER3 alone show low affinities for heregulin, whereas cells transfected with both HER2 and HER3 show higher affinities.

This observation correlates with the "receptor cross-talking" described previously by Kokai *et al.*, *Cell* 58:287-292 (1989); Stern *et al.*, *EMBO J.* 7:995-1001 (1988); and King *et al.*, 4:13-18 (1989). These researchers found that binding of EGF to the EGFR resulted in activation of the EGFR kinase domain and cross-phosphorylation of p185^{HER2}. This is believed to be a result of ligand-induced receptor heterodimerization and the concomitant cross-phosphorylation of the receptors within the heterodimer (Wada *et al.*, *Cell* 61:1339-1347 (1990)).

Plowman and his colleagues have similarly studied p185^{HER4}/p185^{HER2} activation. They expressed p185^{HER2} alone, p185^{HER4} alone, or the two receptors together in human T lymphocytes and demonstrated that heregulin is capable of stimulating tyrosine phosphorylation of p185^{HER4}, but could only stimulate p185^{HER2} phosphorylation in cells expressing both receptors. Plowman *et al.*, *Nature* 336:473-475 (1993).

The biological role of heregulin has been investigated by several groups. For example, Falls *et al.*, (discussed above) found that ARIA plays a role in myotube differentiation, namely affecting the synthesis and concentration of neurotransmitter receptors in the postsynaptic muscle cells of motor neurons. Corfas and Fischbach demonstrated that ARIA also increases the number of sodium channels in chick muscle. Corfas and Fischbach, *J. Neuroscience*, 13(5): 2118-2125 (1993). It has also been shown that GGFII is mitogenic for subconfluent quiescent human myoblasts and that differentiation of clonal human myoblasts in the continuous presence of GGFII results in greater numbers of myotubes after six days of differentiation (Sklar *et al.*, *J. Cell Biochem.*, Abst. W462, 18D, 540 (1994)). See also WO 94/26298 published November 24, 1994.

Holmes *et al.*, *supra*, found that HRG exerted a mitogenic effect on mammary cell lines (such as SK-BR-3 and MCF-7). The mitogenic activity of GGFs on Schwann cells has also been reported. See, e.g., Brockes *et al.*, *J. Biol. Chem.* 255(18):8374-8377 (1980); Lemke and Brockes, *J. Neurosci.* 4:75-83 (1984 Lemke and Brockes, *J. Neurosci.* 4:75-83 (1984); Brockes *et al.*, *Ann. Neurol.* 20(3):317-322 (1986); Brockes, J., *Methods in Enzym.*, 147: 217-225 (1987) and Marchionni *et al.*, *supra*. Schwann cells constitute

87:6378-6382 (1990). However, early efforts to select high affinity fusion phage failed, presumably due to the polyvalence of the phage particles. This problem was solved with the development of a "monovalent" phage display system in which the fusion protein is expressed at a low level from a phagemid and a helper phage provides a large excess of wild-type coat protein. Bass *et al., Proteins* 8:309-314 (1990); Lowman *et al., Biochem.* 30:10832-10838 (1991). Monovalent phage display can be used to generate and screen a large number of variant polypeptides to isolate those that bind with high affinity to a target of interest.

Approximately 50,000 infants are born in the United States every year with birth weights, less than 1.5 kg. About two thirds of these very low birth weight infants have evidence of pulmonary immaturity manifested as respiratory distress shortly after birth. The majority of these infants require mechanical ventilation. Respiratory distress syndrome, caused by insufficient pulmonary surfactant production, as well as structural immaturity of the lung, is responsible for respiratory difficulties observed in these prematurely born neonates. Well developed alveoli are necessary to provide efficient oxygen transfer from the air-liquid interface of the lung to the systemic circulation. Surfactant proteins are critical in reducing the alveolar surface tension at low lung volumes and preventing alveolar collapse.

A need continues to exist for a method of treatment for respiratory distress syndrome and other diseases associated with immature lung development and low lung surfactant production.

SUMMARY OF THE INVENTION

In general an object of the invention is to provide a method of inducing epithelial cell growth and development for the purpose of promoting repair and healing of tissue damage or injury.

Accordingly, one object of this invention is to provide a method of treating respiratory distress syndrome in patients, primarily human patients, in need of such treatment. A further object is to provide a method of inducing lung epithelial cell growth and development. A further object is to provide a method of increasing lung surfactant protein A production in the lung of persons with impaired oxygen transfer in the lung alveoli. This invention is useful in treating infants/neonates with respiratory distress as well as youth and adults with poor lung function due to lung injury or damage.

In one aspect of this invention, it has now been discovered that these objects and the broader objective of treating conditions associated with epithelial cell damage and injury are achieved by administering to a patient in need of such treatment an effective amount of a heregulin ligand, preferably a polypeptide or fragment thereof. These heregulin (HRG) polypeptides, include HRG-α, HRG-β1, HRG-β2, HRG-β3 and other HRG polypeptides which cross-react with antibodies directed against these family members and/or which are substantially homologous as defined below and includes HRG variants such as N-terminal and C-terminal fragments thereof. A preferred HRG is the ligand disclosed in Fig. 1A - 1D and further designated HRG-α. Other preferred HRGs are the ligands disclosed in Figure 2A - 2E, and designated HRG-β1; disclosed in Figure 3A - 3E designated HRG-β2; and disclosed in Figure 4A - 4C designated HRG-β3.

In another aspect, the invention provides a method in which HRG agonist antibodies are administered to achieve the objects of the invention. In this embodiment, HER2/HER3 or fragments thereof (which also may be synthesized by *in vitro* methods) are fused (by recombinant expression or an *in vitro* peptidyl bond) to an immunogenic polypeptide and this fusion polypeptide, in turn, is used to raise antibodies against a HER2/HER3 epitope. Agonist antibodies are recovered from the serum of immunized animals. Alternatively, monoclonal antibodies are prepared from *in vitro* cells or *in vivo* immunized animals in conventional fashion. If desired, the agonist antibodies may be obtained by phage display selection from a phage library of antibodies or antibody fragments. Preferred antibodies identified by routine screening will bind to the receptor, but will not substantially cross-react with any other known ligands such as EGF, and will activate the HER receptors HER2, HER3 and/or HER4. In addition, antibodies may be selected that are

ID NO:12) of γ-HRG obtained as described in U.S. Serial No. 08/891,845. The hydrophobic regi n is underlined. The EGF-like domain is shaded, cysteine residues in the EGF-like domain are circled. N-linked glycosylation sites are marked above the nucleic acid sequence with a ( ).

Figure 8 shows the cDNA sequence (SEQ ID NO:13) and amin acid sequence (SEQ ID NO:14) of SMDF obtained as described in U.S. Serial No. 08/339,517. An EGF-like domain and the apolar and uncharged domains (*i.e.* "apolar I" consisting of residues from about 48-62 and "apolar II" consisting of residues from about 76-100) are underlined. Cysteines in the EGF-like domain and in the "cysteine knot" in the unique N-terminal domain ("NTD-cys knot") are boxed. The stop codon is denoted by the letter "O".

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

HRG ligands, in particular polypeptides and agonist antibodies thereof, have affinity for and stimulate the HER2, HER3 and/or HER4 receptors or combinations thereof in autophosphorylation. Included within the definition of HRG ligands, in addition to HRG-α, HRG-β1, HRG-β2, HRG-β3 and HRG-β2-like, are other polypeptides binding to the HER2, HER3 and/or HER4 receptor, which bear substantial amino acid sequence homology to HRG-α or HRG-β1. Such additional polypeptides fall within the definition of HRG as a family of polypeptide ligands that bind to the HER2, HER3 and/or HER4 receptors.

Heregulin polypeptides bind with varying affinities to the HER2, HER3 and/or HER4 receptors. Generally, the HER3 and HER4 receptors are bound with high affinity. It is also known that heterodimerization of HER2 with HER3 and of HER2 with HER4 occurs with subsequent receptor cross-phosphorylation as described above. In the present invention, epithelial cell growth and/or proliferation is induced when a heregulin protein interacts and binds with an individual receptor molecule or a receptor dimer such that receptor phosphorylation is induced. Binding and activation of HER2, HER3, HER4 or combinations thereof, therefore, is meant to include activation of any form of the receptor necessary for receptor activation and biologic function including monomeric receptor and dimeric receptor forms. Dimeric receptor forms may be referred to below, for example, as HER2/HER3, HER2/HER4, and HER3/HER4.

I. DEFINITIONS

In general, the following words or phrases have the indicated definition when used in the description, examples, and claims.

"Heregulin" (HRG) ligand is defined herein to be any isolated ligand, preferably a polypeptide sequence which possesses a biological property of a naturally occurring HRG polypeptide. Ligands within the scope of this invention include the NDF, ARIA and GGF growth factor heregulin proteins identified above as well as the SMDF and HRG polypeptides discussed in detail herein. These isolated NDF, ARIA and GGF heregulin polypeptides are well known in the art. HRG includes the polypeptides shown in Figs. 1A-1D, 2A-2E, 3A-3E, 4A-4C, 5A-5D, 6A-6C, 7A-7C and 8 and mammalian analogues thereof. Included are HRG variants such as the γ-HRG described in WO 98/02541, published 22 January 1998; the variants described in WO 98/35036, published 13 August 1998; and the SMDF variants described in US Patent N0. 5,770,567 granted 23 June 1998 (WO 96/15244). These applications are incorporated herein in their entirety. These variants can be prepared by the methods described below, optionally together with alanine scanning and phage display techniques known in the art. Cunningham and Wells, Science 244:1081-85 (1989); Bass et al., Proteins 8:309-14 (1990); Lowman et al., Biochem. 30:10832-38 (1991).

The term a "normal" epithelial cell means an epithelial cell which is not transformed, i.e., is non-cancerous and/or non-immortalized. Further, the normal epithelial cell is preferably not aneupl id. Aneuploidy exists when the nucleus of a cell does not contain an exact multiple f the haploid number of chromosomes, one or more chrom somes being present in greater or lesser number than the rest. Typical properties of transformed cells which fall outside the scope of this invention include the ability to form

in its native conformation generally is HRG as found in nature which has not been denatured by chaotropic agents, heat or other treatment that substantially modifies the three dimensional structure of HRG as determined, for example, by migration on nonreducing, nondenaturing sizing gels. Antibody used in this determination may be rabbit polyclonal antibody raised by formulating native HRG from a non-rabbit species in Freund's complete adjuvant, subcutaneously injecting the formulation, and boosting the immune response by intraperitoneal injection of the formulation until the titer of anti-HRG antibody plateaus.

Ordinarily, biologically or antigenically active HRG will have an amino acid sequence having at least 75% amino acid sequence identity with a given HRG sequence, more preferably at least 80%, even more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to an HRG sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with HRG residues in the HRG of Fig. 6A-6C, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into HRG sequence shall be construed as affecting homology.

Thus, the biologically active and antigenically active HRG polypeptides that are the subject of this invention include each entire HRG sequence; fragments thereof having a consecutive sequence of at least 5, 10, 15, 20, 25, 30 or 40 amino acid residues from HRG sequence; amino acid sequence variants of HRG sequence wherein an amino acid residue has been inserted N- or C-terminal to, or within, HRG sequence or its fragment as defined above; amino acid sequence variants of HRG sequence or its fragment as defined above has been substituted by another residue. HRG polypeptides include those containing predetermined mutations by, e.g., site-directed or PCR mutagenesis, and other animal species of HRG polypeptides such as rabbit, rat, porcine, non-human primate, equine, murine, and ovine HRG and alleles or other naturally occurring variants of the foregoing and human sequences; derivatives of HRG or its fragments as defined above wherein HRG or its fragments have been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope); glycosylation variants of HRG (insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of appropriate amino acid); and soluble forms of HRG, such as HRG-GFD or those that lack a functional transmembrane domain.

"Isolated" means a ligand, such as HRG, which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for HRG, and may include proteins, hormones, and other substances. In preferred embodiments, HRG will be purified (1) to greater than 95% by weight of protein as determined by the Lowry method or other validated protein determination method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of the best commercially available amino acid sequenator marketed on the filing date hereof, or (3) to homogeneity by SDS-PAGE using Coomassie blue or, preferably, silver stain. Isolated HRG includes HRG in situ within recombinant cells since at least ne component of HRG natural environment will not be present. Isolated HRG includes HRG from one species in a recombinant cell culture of another species since HRG in such circumstances will be devoid of source polypeptides. Ordinarily, however, isolated HRG will be prepared by at least one purification step.

In accordance with this invention, HRG nucleic acid is RNA or DNA containing greater than ten bases that encodes a biologically or antigenically active HRG, is complementary to nucleic acid sequence encoding such HRG, or hybridizes to nucleic acid sequence encoding such HRG and remains stably bound to it under stringent conditions.

Preferably, HRG nucleic acid encodes a polypeptide sharing at least 75% sequence identity, more preferably at least 80%, still more preferably at least 85%, even more preferably at 90%, and most preferably

"Restriction enzyme digestion" of DNA refers to catalytic cleavage of the DNA with an enzyme that acts only at certain locations in the DNA. Such enzymes are called restriction endonucleases, and the sites for which each is specific is called a restriction site. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements as established by the enzyme suppliers are used. Restriction enzymes commonly are designated by abbreviations composed of a capital letter followed by other letters representing the microorganism from which each restriction enzyme originally was obtained, and then a number designating the particular enzyme. In general, about 1 mg of plasmid or DNA fragment is used with about 1-2 units of enzyme in about 20 ml of buffer solution. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation of about 1 hour at 37°C is ordinarily used, but may vary in accordance with the supplier's instructions. After incubation, protein or polypeptide is removed by extraction with phenol and chloroform, and the digested nucleic acid is recovered from the aqueous fraction by precipitation with ethanol. Digestion with a restriction enzyme may be followed with bacterial alkaline phosphatase hydrolysis of the terminal 5' phosphates to prevent the two restriction cleaved ends of a DNA fragment from "circularizing" or forming a closed loop that would impede insertion of another DNA fragment at the restriction site. Unless otherwise stated, digestion of plasmids is not followed by 5' terminal dephosphorylation. Procedures and reagents for dephosphorylation are conventional as described in sections 1.56-1.61 of Sambrook *et al.*, (*Molecular Cloning: A Laboratory Manual* New York: Cold Spring Harbor Laboratory Press, 1989).

"Recovery" or "isolation" of a given fragment of DNA from a restriction digest means separation of the digest on polyacrylamide or agarose gel by electrophoresis, identification of the fragment of interest by comparison of its mobility versus that of marker DNA fragments of known molecular weight, removal of the gel section containing the desired fragment, and separation of the gel from DNA. This procedure is known generally. For example, see Lawn *et al.*, *Nucleic Acids Res.* 9:6103-6114 (1981), and Goeddel *et al.*, *Nucleic Acids Res.* 8:4057 1980).

"Northern analysis" is a method used to identify RNA sequences that hybridize to a known probe such as an oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as ^{32}P, or by biotinylation, or with an enzyme. The RNA to be analyzed is usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook *et al.*, *supra.*

"Ligation" refers to the process of forming phosphodiester bonds between two nucleic acid fragments. To ligate the DNA fragments together, the ends of the DNA fragments must be compatible with each other. In some cases, the ends will be directly compatible after endonuclease digestion. However, it may be necessary to first convert the staggered ends commonly produced after endonuclease digestion to blunt ends to make them compatible for ligation. To blunt the ends, the DNA is treated in a suitable buffer for at least 15 minutes at 15°C with about 10 units of the Klenow fragment of DNA polymerase I or T4 DNA polymerase in the presence of the four deoxyribonucleotide triphosphates. The DNA is then purified by phenol-chloroform extraction and ethanol precipitation. The DNA fragments that are to be ligated together are put in solution in about equimolar amounts. The solution will also contain ATP, ligase buffer, and a ligase such as T4 DNA ligase at about 10 units per 0.5 mg of DNA. If the DNA is to be ligated into a vector, the vector is first linearized by digestion with the appropriate restriction endonuclease(s). The linearized fragment is then treated with bacterial alkaline phosphatase, or calf intestinal phosphatase to prevent self-ligation during the ligation step.

"Preparation" of DNA from cells means isolating the plasmid DNA from a culture of the host cells. Commonly used methods for DNA preparation are the large and small-scale plasmid preparations described

"Protein microsequencing" was accomplished based upon the following procedures. Proteins from the final HPLC step were either sequenced directly by automated Edman degradation with a model 470A Applied Biosystems gas phase sequencer equipped with a 120A PTH amino acid analyzer or sequenced after digestion with various chemicals or enzymes. PTH amino acids were integrated using the CHROMPERFECT data system (Justice Innovations, Palo Alto, CA). Sequence interpretation was performed on a VAX 11/785 Digital Equipment Corporation computer as described (Henzel *et al.*, *J. Chromatography* 404:41-52 (1987)). In some cases, aliquots of the HPLC fractions were electrophoresed on 5-20% SDS polyacrylamide gels, electrotransferred to a PVDF membrane (PROBLOTT, ABI, Foster City, CA) and stained with Coomassie Brilliant Blue (Matsudaira, P., *J. Biol. Chem.* 262:10035-10038, 1987). The specific protein was excised from the blot for N terminal sequencing. To determine internal protein sequences, HPLC fractions were dried under vacuum (SPEEDVAC), resuspended in appropriate buffers, and digested with cyanogen bromide, the lysine-specific enzyme Lys-C (Wako Chemicals, Richmond, VA) or Asp-N (Boehringer Mannheim, Indianapolis, Ind.). After digestion, the resultant peptides were sequenced as a mixture or were resolved by HPLC on a C4 column developed with a propanol gradient in 0.1% TFA before sequencing as described above.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteine(s) from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ϵ, γ, and μ, respectively. The subunit structures and three-dimensional

antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

The expression "linear antibodies" when used throughout this application refers to the antibodies described in Zapata *et al. Protein Eng.* 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

II. USE AND PREPARATION OF HRG SEQUENCES

H. PREPARATION OF HRG SEQUENCES, INCLUDING VARIANTS

The system to be employed in preparing HRG sequence will depend upon the particular HRG sequence selected. If the sequence is sufficiently small HRG may be prepared by *in vitro* polypeptide synthetic methods. Most commonly, however, HRG will be prepared in recombinant cell culture using the host-vector systems described below. Suitable HRG includes any biologically active and antigenetically active HRG.

In general, mammalian host cells will be employed, and such hosts may or may not contain post-translational systems for processing HRG preprosequences in the normal fashion. If the host cells contain such systems then it will be possible to recover natural subdomain fragments such as HRG-GFD from the cultures. If not, then the proper processing can be accomplished by transforming the hosts with the required enzyme(s) or by supplying them in an *in vitro* method. However, it is not necessary to transform cells with the complete prepro or structural genes for a selected HRG when it is desired to only produce fragments of HRG sequences such as an HRG-GFD. For example, a start codon is ligated to the 5' end of DNA encoding an HRG-GFD, this DNA is used to transform host cells and the product expressed directly as the Met N-terminal form (if desired, the extraneous Met may be removed *in vitro* or by endogenous N-terminal demethionylases). Alternatively, HRG-GFD is expressed as a fusion with a signal sequence recognized by the host cell, which will process and secrete the mature HRG-GFD as is further described below. Amino acid sequence variants of native HRG-GFD sequences are produced in the same way.

HRG sequences located between the first N-terminal mature residue and the first N-terminal residue of HRG-GFD sequence, termed HRG-NTD, may function at least in part as an unconventional signal sequence or as a normally circulating carrier/precursor for HRG-GFD having unique biological activity. HRG-NTD is produced in the same fashion as the full length molecule but from expression of DNA in which a stop codon is located at the C-terminus of HRG-NTD. In addition, HRG variants are expressed from DNA encoding protein in which both the GFD and NTD domains are in their proper orientation but which contain an amino acid insertion, deletion or substitution at the GFD-NTD cleavage site (located within the sequence VKC) which inhibits or prevents proteolytic cleavage of the NTD-GFD joining site *in vivo*, and wherein a stop codon is positioned at the 3' end of the GFD- encoding sequence. In an example of this group f variants (termed HRG-NTDXGFD), (1) the lysine residue found in the NTD-GFD joining sequence VKC is deleted or (preferably) substituted by another residue other than arginyl such as histidyl, alanyl, threonyl or seryl and (2) a stop codon is introduced in the sequence RCT or RCQ in place of cysteinyl, or threonyl (for HRG-α) or glutaminyl (for HRG-β).

screening genomic DNA libraries include, but are not limited to, oligonucleotides; cDNAs or fragments thereof that encode the same or a similar gene; and/or homologous genomic DNAs or fragments thereof. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al., supra.*

An alternative means to isolate the gene encoding HRG-α is to use polymerase chain reaction (PCR) methodology as described in section 14 of Sambrook *et al., supra.* This method requires the use of oligonucleotide probes that will hybridize to HRG-α. Strategies for selection of oligonucleotides are described below.

Another alternative method for obtaining the gene of interest is to chemically synthesize it using one of the methods described in Engels *et al.* (*Agnew. Chem. Int. Ed. Engl.*, 28: 716-734,1989. These methods include triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues, preferably human breast, colon, salivary gland, placental, fetal, brain, and carcinoma cell lines. Other biological sources of DNA encoding an heregulin-like ligand include other mammals and birds. Among the preferred mammals are members of the following orders: bovine, ovine, equine, murine, and rodentia.

The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The actual nucleotide sequence(s) may, for example, be based on conserved or highly homologous nucleotide sequences or regions of HRG-α. The oligonucleotides may be degenerate at one or more positions. The use of degenerate oligonucleotides may be of particular importance where a library is screened from a species in which preferential codon usage in that species is not known. The oligonucleotide must be labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P-labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Of particular interest is HRG-α nucleic acid that encodes a full-length polypeptide. In some preferred embodiments, the nucleic acid sequence includes the native HRG-α signal sequence. Nucleic acid having all the protein coding sequence is obtained by screening selected cDNA or genomic libraries, and, if necessary, using conventional primer extension procedures as described in section 7.79 of Sambrook *et al., supra,* to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

HRG-α encoding DNA of Figures 1A-1D may be used to isolate DNA encoding the analogous ligand from other animal species via hybridization employing the methods discussed above. The preferred animals are mammals, particularly bovine, ovine, equine, feline, canine and rodentia, and more specifically rats, mice and rabbits.

B. Amino Acid Sequence Variants of Heregulin

Amino acid sequence variants of HRG are prepared by introducing appropriate nucleotide changes into HRG DNA, or by *in vitro* synthesis of the desired HRG polypeptide. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence shown for human HRG sequences. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. Excluded from the

represent naturally occurring alleles (which will not require manipulation of HRG DNA) or predetermined mutant forms made by mutating the DNA, either to arrive at an allele or a variant not found in nature. In general, the location and nature of the mutation chosen will depend upon HRG characteristic to be modified. Obviously, such variations that, for example, convert HRG into a known receptor ligand, are not included within the scope of this invention, nor are any other HRG variants or polypeptide sequences that are not novel and unobvious over the prior art.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues, and typically about 1 to 5 are contiguous. Deletions may be introduced into regions of low homology with other EGF family precursors to modify the activity of HRG. Deletions from HRG in areas of substantial homology with other EGF family sequences will be more likely to modify the biological activity of HRG more significantly. The number of consecutive deletions will be selected so as to preserve the tertiary structure of HRG in the affected domain, e.g., cysteine crosslinking, beta-pleated sheet or alpha helix.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within HRG sequence) may range generally from about 1 to 10 residues, more preferably 1 to 5, and most preferably 1 to 3. Examples of terminal insertions include HRG with an N-terminal methionyl residue (an artifact of the direct expression of HRG in bacterial recombinant cell culture), and fusion of a heterologous N-terminal signal sequence to the N-terminus of HRG to facilitate the secretion of mature HRG from recombinant host cells. Such signal sequences generally will be obtained from, and thus be homologous to, the intended host cell species. Suitable sequences include STII, tPA or lpp for *E. coli*, alpha factor for yeast, and viral signals such as herpes gD for mammalian cells.

Other insertional variants of HRG include the fusion to the N- or C-terminus of HRG of an immunogenic polypeptide, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, bovine serum albumin, and chemotactic polypeptides. C-terminal fusions of HRG-ECD with proteins having a long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, as described in WO 89/02922, published 6 April 1989 are contemplated.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in HRG molecule removed and a different residue inserted in its place. The sites f greatest interest for substitutional mutagenesis include sites identified as the active site(s) of HRG, and sites where the amino acids found in HRG ligands from various species are substantially different in terms of side-chain bulk, charge, and/or hydrophobicity. A likely sub-domain of HRG-GFD having biological activity as a growth factor is the C-terminal segment, in particular within the sequence about from glycine 218 to valine 226 (HRG-α), and glycine 218 to lysine 228/serine 228 (HRG-β) based upon analogy to the EGF sub-sequence found to have EGF activity.

Other sites of interest are those in which particular residues of HRG-like ligands obtained from various species are identical. These positions may be important for the biological activity of HRG. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

In another embodiment, any methionyl residue other than the starting methionyl residue f the signal sequence, or any residue located within about three residues N- or C-terminal to each such methionyl residue, is substituted by another residue (preferably in accord with Table 1) or deleted. Alternatively, about 1-3 residues are inserted adjacent to such sites.

Any cysteine residues not involved in maintaining the proper conformation of HRG also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

Sites particularly suited for substitutions, deletions or insertions, or use as fragments, include, numbered from the N-terminus of HRG-α of Figure 1A - 1D:

1) potential glycosaminoglycan addition sites at the serine-glycine dipeptides at 42-43, 64-65, 151-152;

2) potential asparagine-linked glycosylation at positions 164, 170, 208 and 437, sites (NDS) 164-166, (NIT) 170-172, (NTS) 208-210, and NTS (609-611);

3) potential O-glycosylation in a cluster of serine and threonine at 209-218;

4) cysteines at 226, 234, 240, 254, 256 and 265;

5) transmembrane domain at 287-309;

6) loop 1 delineated by cysteines 226 and 240;

7) loop 2 delineated by cysteines 234 and 254;

8) loop 3 delineated by cysteines 256 and 265; and

9) potential protease processing sites at 2-3, 8-9, 23-24, 33-34, 36-37, 45-46, 48-49, 62-63, 66-67, 86-87, 110-111, 123-124, 134-135, 142-143, 272-273, 278-279 and 285-286;

Analogous regions in HRG-β1 may be determined by reference to its' sequence. The analogous HRG-β1 amino acids may be mutated or modified as discussed above for HRG-α. Analogous regions in HRG-β2 may also be determined by reference to its' sequence. The analogous HRG-β2 amino acids may be mutated or modified as discussed above for HRG-α or HRG-β1. Analogous regions in HRG-β3 may be determined by reference to its' sequence. Further, the analogous HRG-β3 amino acids may be mutated or modified as discussed above for HRG-α, HRG-β1, or HRG-β2.

Another HRG variant is γ-HRG (or gamma-heregulin). γ-HRG is any polypeptide sequence that possesses at least one biological property of native sequence γ-HRG having SEQ ID NO:11. The biological property of this variant is the same as for HRG noted above. This variant encompasses not only the polypeptide isolated from a native γ-HRG source such as human MDA-MB-175 cells or from another source, such as another animal species, but also the polypeptide prepared by recombinant or synthetic methods. It also includes variant forms including functional derivatives, allelic variants, naturally occurring isoforms and analogues thereof. Sometimes the γ-HRG is "native γ-HRG" which refers to endogenous γ-HRG polypeptide which has been isolated from a mammal. The γ-HRG can also be "native sequence γ-HRG" insofar as it has the same amino acid sequence as a native γ-HRG (*e.g.* human γ-HRG shown in Fig. 7A-7C). Amino acid sequence variants of the native sequence are prepared by introducing appropriate nucleotide changes into the native sequence DNA, or by *in vitro* synthesis of the desired polypeptide. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence shown for the human protein in Fig. 7A-7C as generally described above for other HRG. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the native sequence, such as changing the number or position of O-linked

A183G, K185E, E186R, K187E, T188G, F197Y, M198R, D201T;
A183G, K185E, E186R, K187E, T188G, P205Y, S206G, R207Y, Y208L, L209M;
A183G, K185E, E186R, K187E, T188G, F197Y, M198R, D201T, P205Y, S206G, R207Y, Y208L, L209M;
A183G, K185E, E186R, K187E, T188G, M226I;
F197Y, M198R, D201T, P205Y, S206G, R207Y, Y208L, L209M;
F197Y, M198R, D201T, P205Y, S206G, R207Y, Y208L, L209M, M226I;
F197Y, M198R, D201T, M226I;
A183G, K185E, E186R, K187E, T188G, F197Y, M198R, D201T, M226I;
A183G, K185E, E186R, K187E, T188G, P205Y, S206G, R207Y, Y208L, L209M, M226I;
A183G, K185E, E186R, K187E, T188G, F197Y, M198R, D201T, P205Y, S206G, R207Y, Y208L, L209M, M226I;
F197Y, M198R, D201T, P205Y, S206G, R207Y, Y208L, L209M, N223H, M226I; and
A183G, K185E, E186R, K187E, T188G, F197Y, M198R, D201T, P205Y, S206G, R207Y, Y208L, L209M, N223H, M226I.

In addition to including one or more of the amino acid substitutions disclosed herein, the heregulin variant can have one or more other modifications, such as an amino acid substitution, an insertion of at least one amino acid, a deletion of at least one amino acid, or a chemical modification. For example, the invention provides a heregulin variant that is a fragment. In a variation of this embodiment, the fragment includes residues corresponding to a portion of human heregulin-β1 extending from about residue 175 to about residue 230 (i.e., the EGF-like domain). For example, the fragment can extend from residue 177 to residue 244 and may be prepared by recombinant techniques (rHRGβ1-177-244).

DNA encoding amino acid sequence variants of HRG is prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of HRG. These techniques may utilize HRG nucleic acid (DNA or RNA), or nucleic acid complementary to HRG nucleic acid.

Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion, and insertion variants of HRG DNA. This technique is well known in the art as described by Adelman *et al.*, *DNA*, 2: 183 (1983). Briefly, HRG DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of HRG. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in HRG DNA.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea *et al.* (*Proc. Natl. Acad. Sci. USA*, 75: 5765,1978).

Single-stranded DNA template may also be generated by denaturing double-stranded plasmid (or other) DNA using standard techniques.

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the ligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A

primers is designed to overlap the position of the mutation and to contain the mutation; the sequence of the other primer must be identical to a stretch of sequence of the opposite strand of the plasmid, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone.

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using standard DNA technology. Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

In a specific example of PCR mutagenesis, template plasmid DNA (1mg) is linearized by digestion with a restriction endonuclease that has a unique recognition site in the plasmid DNA outside of the region to be amplified. Of this material, 100 ng is added to a PCR mixture containing PCR buffer, which contains the four deoxynucleotide tri-phosphates and is included in the GENEAMP kits (obtained from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA), and 25 pmole of each oligonucleotide primer, to a final volume of 50 ml. The reaction mixture is overlaid with 35 ml mineral oil. The reaction is denatured for 5 minutes at 100°C, placed briefly on ice, and then 1 ml *Thermus aquaticus (Taq)* DNA polymerase (5 units/ml, purchased from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA) is added below the mineral oil layer. The reaction mixture is then inserted into a DNA Thermal Cycler (purchased from Perkin-Elmer Cetus) programmed as follows:

2 min. 55°C,
30 sec. 72°C, then 19 cycles of the following:
30 sec. 94°C,
30 sec. 55°C, and
30 sec. 72°C.

At the end of the program, the reaction vial is removed from the thermal cycler and the aqueous phase transferred to a new vial, extracted with phenol/chloroform (50:50:vol), and ethanol precipitated, and the DNA is recovered by standard procedures. This material is subsequently subjected to the appropriate treatments for insertion into a vector.

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells *et al.* (*Gene*, 34: 315,1985). The starting material is the plasmid (or other vector) comprising HRG DNA to be mutated. The codon(s) in HRG DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in HRG DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated HRG DNA sequence.

C. Insertion of DNA into a Cloning Vehicl

The cDNA or genomic DNA encoding native or variant HRG is inserted into a replicable vector for further cloning (amplification of the DNA) or f r expression. Many vectors are available, and selection of

encoding HRG is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise HRG DNA. DNA can be amplified by PCR and directly transfected into the host cells without any replication component.

(iii) Selection Gene Compon nt

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern *et al.*, *J. Molec. Appl. Genet.* 1: 327,1982), mycophenolic acid (Mulligan *et al.*, *Science* 209: 1422,1980) or hygromycin (Sugden *et al.*, *Mol. Cell. Biol.* 5: 410-413,1985). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up HRG nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes HRG. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of HRG are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77: 4216, 1980. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding HRG. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding HRG, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418 (see U.S. Pat. No. 4,965,199).

A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, *Nature*, 282: 39, 1979; Kingsman *et al.*, *Gene*, 7: 141, 1979; or Tschemper *et al.*, *Gene*, 10: 157, 1980). The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan; for example, ATCC No. 44076 or PEP4-1 (Jones, *Genetics*, 85: 12, 1977). The presence of the *trp*1 lesion in the yeast host cell genome then provides an effective environment for detecting

promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with HRG sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication (Fiers *et al.*, *Nature*, 273:113 (1978); Mulligan and Berg, *Science*, 209: 1422-1427 (1980); Pavlakis *et al.*, *Proc. Natl. Acad. Sci. USA*, 78: 7398-7402 (1981)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a *Hind*III E restriction fragment (Greenaway *et al.*, *Gene*, 18: 355-360 (1982)). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, *Nature*, 295: 503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; Reyes *et al.*, *Nature*, 297: 598-601 (1982) on expression of human b-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus; Canaani and Berg, *Proc. Natl. Acad. Sci. USA*, 79: 5166-5170 (1982) on expression of the human interferon b1 gene in cultured mouse and rabbit cells; and Gorman *et al.*, *Proc. Natl. Acad. Sci. USA*, 79: 6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

(v) Enhancer Element Component

Transcription of a DNA encoding HRG of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10-300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins *et al.*, *Proc. Natl. Acad. Sci. USA*, 78: 993, 1981) and 3' (Lusky *et al.*, *Mol. Cell Bio.*, 3: 1108, 1983) to the transcription unit, within an intron (Banerji *et al.*, *Cell*, 33: 729, 1983) as well as within the coding sequence itself (Osborne *et al.*, *Mol. Cell Bio.*, 4: 1293, 1984). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, a-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers (see also Yaniv, *Nature*, 297: 17-18 (1982)) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to HRG DNA, but is preferably located at a site 5' from the promoter.

(vi) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding HRG. The 3' untranslated regions also include transcription termination sites.

Construction of suitable vectors containing one or more of the above listed components the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing *et al.*, *Nucleic Acids Res.* 9: 309 (1981) or by the method of Maxam *et al.*, *Methods in Enzymology* 65: 499 (1980).

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain HRG DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding HRG is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express HRG DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences (Depicker *et al.*, *J. Mol. Appl. Gen.*, 1: 561 (1982)). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue (see EP 321,196, published 21 June 1989).

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years (*Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973)). Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.*, 36: 59, 1977); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77: 4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23: 243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.*, 383: 44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *supra*, is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene*, 23: 315 (1983) and WO 89/05859, published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30-16.37 of Sambrook *et al*, *supra*, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216, issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.*, 130: 946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 76: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or protoplast fusion may also be used.

E. Culturing the Host Cells

Prokaryotic cells used to produce HRG polypeptide of this invention are cultured in suitable media as described generally in Sambrook *et al.*, *supra*.

prepared against a native HRG polypeptide or against a synthetic peptide based on the DNA sequences provided herein as described further below.

G. Purification of The Heregulin Polypeptides

HRG is recovered from a cellular membrane fraction. Alternatively, a proteolytically cleaved or a truncated expressed soluble HRG fragment or subdomain are recovered from the culture medium as a soluble polypeptide. A HRG is recovered from host cell lysates when directly expressed without a secretory signal.

When HRG is expressed in a recombinant cell other than one of human origin, HRG is completely free of proteins or polypeptides of human origin. However, it is desirable to purify HRG from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to HRG. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The membrane and soluble protein fractions are then separated. HRG is then be purified from both the soluble protein fraction (requiring the presence of a protease) and from the membrane fraction of the culture lysate, depending on whether HRG is membrane bound. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica, heparin SEPHAROSE or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; and gel filtration using, for example, SEPHADEX G-75.

HRG variants in which residues have been deleted, inserted or substituted are recovered in the same fashion as the native HRG, taking account of any substantial changes in properties occasioned by the variation. For example, preparation of a HRG fusion with another protein or polypeptide, e.g., a bacterial r viral antigen, facilitates purification; an immunoaffinity column containing antibody to the antigen can be used to adsorb the fusion. Immunoaffinity columns such as a rabbit polyclonal anti-HRG column can be employed to absorb HRG variant by binding it to at least one remaining immune epitope. A protease inhibitor such as phenylmethylsulfonylfluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native HRG may require modification to account for changes in the character of HRG variants or upon expression in recombinant cell culture.

H. Covalent Modifications of HRG

Covalent modifications of HRG polypeptides are included within the scope of this invention. Both native HRG and amino acid sequence variants of HRG optionally are covalently modified. One type of covalent modification included within the scope of this invention is a HRG polypeptide fragment. HRG fragments, such as HRG-GDF, having up to about 40 amino acid residues are conveniently prepared by chemical synthesis, or by enzymatic or chemical cleavage of the full-length HRG polypeptide or HRG variant polypeptide. Other types of covalent modifications of HRG or fragments thereof are introduced into the molecule by reacting targeted amino acid residues of HRG or fragments thereof with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Glycosylation sites are added to HRG by altering its amino acid sequence to contain one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to HRG (for O-linked glycosylation sites). For ease, HRG is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding HRG at preselected bases such that codons are generated that will translate into the desired amino acids.

Chemical or enzymatic coupling of glycosides to HRG increases the number of carbohydrate substituents. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that is capable of N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330, published 11 September 1987, and in Aplin and Wriston (*CRC Crit. Rev. Biochem.*, pp. 259-306 (1981)).

Carbohydrate moieties present on an HRG also are removed chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin *et al.* (*Arch. Biochem. Biophys.*, 259:52 (1987)) and by Edge *et al.* (*Anal. Biochem.*, 118:131 (1981)). Carbohydrate moieties are removed from HRG by a variety of endo- and exo- glycosidases as described by Thotakura *et al.* (*Meth. Enzymol.*, 138:350 (1987)).

Glycosylation also is suppressed by tunicamycin as described by Duskin *et al.* (*J. Biol. Chem.*, 257:3105 (1982)). Tunicamycin blocks the formation of protein-N-glycoside linkages.

HRG may also be modified by linking HRG to various nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in U.S. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

One preferred way to increase the *in vivo* circulating half life of non-membrane bound HRG is to conjugate it to a polymer that confers extended half-life, such as polyethylene glycol (PEG). (Maxfield, *et al*, Polymer 16,505-509 (1975); Bailey, F. E., *et al*, in Nonionic Surfactants (Schick, M. J., ed.) pp.794-821, 1967); (Abuchowski, A. *et al.*, J. Biol. Chem. 252, 3582-3586, 1977; Abuchowski, A. *et al.*, *Cancer Biochem. Biophys.* 7, 175-186, 1984); (Katre, N.V. *et al.*, *Proc. Natl. Acad. Sci.*, 84, 1487-1491, 1987; Goodson, R. *et al. Bio Technology*, 8, 343-346, 1990). Conjugation to PEG also has been reported to have reduced immunogenicity and toxicity (Abuchowski, A. *et al.*, *J. Biol. Chem.*, 252, 3578-3581, 1977).

HRG may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, 16th edition, Osol, A., Ed., (1980).

Those skilled in the art will be capable of screening variants in order to select the optimal variant for the purpose intended. For example, a change in the immunological character of HRG, such as a change in affinity for a given antigen or for the HER2 receptor, is measured by a competitive-type immunoassay using

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-SEPHAROSE, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies will be described in more detail below.

Hybridoma cell lines producing antibodies are identified by screening the culture supernatants for antibody which binds to HER2/HER3 receptors. This is routinely accomplished by conventional immunoassays using soluble receptor preparations or by FACS using cell-bound receptor and labeled candidate antibody. Agonist antibodies are preferably antibodies which stimulate autophosphorylation in the HRG tyrosine autophosphorylation assay described above.

The hybrid cell lines can be maintained in culture *in vitro* in cell culture media. The cell lines of this invention can be selected and/or maintained in a composition comprising the continuous cell line in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody. The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM as the case may be that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g., ethanol or polyethylene glycol precipitation procedures.

Human antibodies may be used and are preferable. Such antibodies can be obtained by using human hybridomas (Cote *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985)). Chimeric antibodies, Cabilly et al., U.S. 4,816,567, (Morrison *et al.*, *Proc. Natl. Acad. Sci.*, 81:6851 (1984); Neuberger *et al.*, *Nature* 312:604 (1984); Takeda *et al.*, *Nature* 314:452 (1985)) containing a murine anti-HER2/HER3 variable region and a human constant region of appropriate biological activity (such as ability to activate human complement and mediate ADCC) are within the scope of this invention, as are humanized antibodies produced by conventional CDR-grafting methods(Riechmann et al., Nature 332:333-327(1988); EP 0328404 A1; EP 02394000 A2).

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (Fab or variable regions fragments) which bypass the generation of monoclonal antibodies are also

Th rapeutic formulations of HRG or ag nist antibody are prepared for storage by mixing the HRG protein having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (*Remington's Pharmaceutical Sciences*, *supra*), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN, PLURONICS or polyethylene glycol (PEG).

HRG or agonist antibody to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The HRG or antibody ordinarily will be stored in lyophilized form or in solution.

Therapeutic HRG or antibody compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of HRG or antibody administration is in accord with known methods, e.g., injection r infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, powder or liquid aerosol administration to the nose or lung or intralesional routes, or by sustained release systems as noted below. The HRG ligand may be administered continuously by infusion or by bolus injection. An agonist antibody is preferably administered in the same fashion, or by administration into the blood stream or lymph.

The HRG, HRG variant or fragment and agonist antibodies may be spray dried or spray freeze dried using known techniques (Yeo et al, Biotech. and Bioeng., 41:341-346 (1993); Gombotz et al, PCT/US90/02421).

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, *J. Biomed. Mater. Res.*, 15: 167-277 (1981) and Langer, *Chem. Tech.*, 12: 98-105 (1982) or poly(vinylalcohol)), polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, *Biopolymers*, 22: 547-556 (1983)), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988). While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release HRG or antibody compositions also include liposomally entrapped HRG or antibody. Liposomes containing HRG or antibody are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82: 3688-3692 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*,

$L^{636}{}_{HER3}$-(TR)-$DKTH^{224}{}_{VH}$; for HER4, $G^{640}{}_{HER4}$-(TR)-$DKTH^{224}{}_{VH}$. The conserved TR sequence is derived from the MI I site. The final expression constructs were in a pRK-type plasmid backbone wherein eukaryotic expression is driven by a CMV promoter (Gorman *et al., DNA Prot. Eng. Tech.* 2:3-10 (1990)).

To obtain protein for *in vitro* experiments, adherent HEK-293 cells were transfected with the appropriate expression plasmids using standard calcium phosphate methods (Gorman *et al., supra* and Huang *et al., Nucleic Acids Res.* 18:937-947 (1990)). Serum-containing media was replaced with serum-free media 15 hours post-transfection and the transfected cells incubated for 5-7 days. The resulting conditioned media was harvested and passed through Protein A columns (1 mL Pharmacia HiTrap™). Purified IgG fusions were eluted with 0.1 M citric acid (pH 4.2) into tubes containing 1 M Tris pH 9.0. The eluted proteins were subsequently dialyzed against PBS and concentrated using Centri-prep-30 filters (Amicon). Glycerol was added to a final concentration of 25% and the material stored at -20 C. Concentrations of material were determined via a Fc-ELISA.

Cell Culture: Human breast cancer cell lines MDA-MB-175, MDA-MB-231, SK-BR-3 and MCF7 were obtained from the American Type Culture Collection and maintained in a 50:50 mixture of F12 Ham's and Dulbecco's modified Eagle medium (DMEM), supplemented with 10% heat inactivated FBS, 2 mM glutamine and 10% penicillin-streptomycin.

Generation and Characterization of cDNA Library: Total RNA was purified from MDA-MB-175 cells using the guanidinium isothiocyanate-cesium chloride procedure (Sambrook *et al., Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, (1989)). Poly $(A)^+$ RNA was isolated using oligo (dT) Dynabeads (DYNAL) as recommended by the supplier. First and second strand syntheses were performed using a Gibco BRL cDNA synthesis kit. λgt10 cDNA recombinants were generated when a cDNA cloning system from Amersham was used. *In vitro* packaging was performed using Gigapack II packaging extract (Stratagene). PstI-XhoI HRGβ3 cDNA fragment (nt 144-618) was labeled by random priming and 1 x 10^6 plaques were screened. Positive clones were confirmed and purified by secondary and tertiary screening. Phage DNA was isolated as a BamHI fragment and subcloned into the corresponding site of pBluescript SK^-. Clone 5 was completely sequenced using the Sequenase version 2.0 DNA sequencing kit (United States Biochemicals, Inc.). Both strands were sequenced.

Bacterial Expression System: A cDNA fragment of clone 5 (nt 1690- 2722) was subcloned into the pET-32 TRX fusion vector (Novagen). This BglII-BglII fragment was inserted into the BamHI site of the pET32a plasmid. The trxγ-HRG (amino acids 455-768) protein expression in *E.coli* was induced as recommended by the supplier.

Purification of Recombinant γ-HRG: *E.coli* cells expressing trxγ-HRG were collected and suspended at 9 ml/g in 50 mM Tris HCL pH 8. Lysozyme was added to a final concentration of 0.2 mg/ml and the solution was stirred on ice for 1 hr. Dnase I (10 μg/ml) and $MgCl_2$ (4 mM) were added. The solution was then sonicated for 30 min and cell pellets collected afterwards. The pellet fraction was dissolved at 250 ml/g in 6 M Gdn HCL, 0.1 M Tris HCL, pH 8.8. Solubilized proteins were sulfitolyzed by adding 1/10 volume of 1 M Na_2SO_3 and 1/10 volume of 0.2 M $Na_2S_4O_6$. The reaction was allowed to proceed for 1.5 hours at room temperature and protein was purified by gel filtration chromatography using a High Load Superdex™ 75 prep grade column (Pharmacia). Refolding was initiated by the addition of 1 mM cysteine, and 10 mM methionine was added as an antioxidant and incubated overnight at room temperature. Protein concentration was determined by quantitative amino acid analysis.

Northern and Southern Hybridization: Total RNA was isolated by the method of Chomczynski *et al. Anal Biochem.* 162:156-159 (1987). Poly $(A)^+$ was isolated using oligo d(T) cellulose columns (Qiagen) as recommended by the supplier. RNA was denatured and size fractioned in a 0.8% formaldehyde/ 1%

seeded in T175 flasks and grown until reaching 70-80% confluency (~ 2.5 x 10^7 cells/ flask). Subsequently, cells were washed with PBS and grown in serum free F12/ DMEM medium for 3-4 days. Medium was then collected, filtered and concentrated using an ultrafiltration cell with YM10 Diaflo ultafiltration membranes (Amicon). γ-HRG was visualized in conditioned medium of MDA-MB-175 cells by Western blot analysis under non reducing conditions. γ-HRG was partially purified by HPLC using a C4 reverse phase column. CHO expressed full length HRGβ1 (lane 1) and semi pure γ-HRG (lane 2) were electrophoresed, blot was probed with HER2/HER4 IgG heterodimers and Western blot was developed. A ~ 64 kDa band could be seen in the lane containing partial purified supernatant whereas CHO expressed full length HRGβ1 migrated as a 45 kDa protein.

Cell Proliferation Assay with Crystal Violet: Tumor cell lines were plated in 96 well plates at following densities: 2 x 10^4 cells/well for MDA-MB-175 and 1 x 10^4 cells/well for SK-BR-3. The media contained 1% FBS and cells were allowed to adhere for 2 hours. Monoclonal antibodies, immunoadhesions (10 μg/ml) or media alone were added and the cells were incubated for 2 hours at 37°C. $rHRG\beta1_{177-244}$ was added at a final concentration of 1 nM, or 100 nM for neutralising the immunoadhesion, and the cells were incubated for 4 days. Monolayers were washed with PBS and stained/fixed with 0.5% crystal violet. Plates were air dried, the dye was eluted with 0.1 M sodium citrate (pH 4.2) in ethanol (50:50) and the absorbance was measured at 540 nm.

Isolation and sequence analysis of γ-HRG: To characterize the heregulin transcript in MDA-MB-175 cells, a λgt 10 cDNA library was constructed with mRNA derived from this cell line. The library was screened with a cDNA probe corresponding to the EGF-like domain and part of the N-terminal sequence of HRGβ3. Various clones were identified. One of the clones which appeared to contain the full length cDNA sequence was isolated and sequenced. Fig.7A-7D shows the nucleotide sequence and the predicted amino acid sequence of γ-HRG. The single open reading frame of 2303 bp starts with an ATG codon at nt 334. This start codon lies in a nucleotide sequence context, which is known to be a potential translation initiation site (Kozak, *Nucleic Acid Research* 15:8125-8148 (1987)). Several termination codons were found upstream of the initiation codon. The stop codon TAG at nt 2637 is followed by the 3' noncoding sequence, which is identical to other HRG isoform sequences and includes a polyadenylation signal followed by an A-rich region. The open reading frame encodes a protein of 768 amino acid residues with a calculated molecular mass of 84.2 kDa.

(d) The selection of HRG-β1 variants containing residues corresponding to the minimal EGF-like domain (HRG-β1 177-228) was conducted using monovalent phage display. For these variants, residue numbers also are expressed, in parentheses, in terms of the position of the residue in the minimal EGF-like domain (i.e., HRG-β1 EGF 1-52).

Variants of HRG-β1 EGF were prepared and selected for binding to HER-3-Ig using monovalent phage display, according to the method of Bass *et al.*, *Proteins* 8:309-314 (1990). As discussed in detail below, an HRG-β1 EGF phagemid vector was prepared, in which HRG-β1 EGF was fused to a C-terminal fragment of the M13 coat protein pIII. Kunkel mutagenesis was performed to introduce stop codons into this vector at sites selected for randomization. This step ensures that the starting vector is incapable of expressing the wild-type polypeptide. Stretches of four to six residues per library were randomized in a linear fashion, except for the six cysteines, Phe189 (HRG-β1 EGF Phe13) and the two most C-terminal residues. Phe189 was not altered because this residue is conserved as an aromatic residue in EGF and TGF-α and forms a stacking interaction with Tyr208 (HRG-β1 EGF Tyr32) Jacobsen *et al.*, *Biochemistry* 35:3402-

Table 4: Library C Variants

	Position in HRG-β1				
Variant No.	191	192	193	194	195
Wild-type	V	N	G	G	E
1, 2, 4, 5, 7-12	.	.	.	.	.
3	.	.	.	.	V
6	.	.	.	.	Q

Table 5: Library D Variants

	Position in HRG-β1				
Variant No.	197	198	199	200	201
Wild-type	F	M	V	K	D
1*, 2*, 8*, 12*	Y	K	.	R	I
3	.	R	.	.	T
4, 5, 7, 9	Y	R	.	.	T
6	Y	.	I	.	Y
10	Y	.	.	.	T
11	M	R	.	R	T

*Variant also contained Met226Ile.

Table 6: Library E Variants

	Position in HRG-β1				
Variant No.	205	206	207	208	209
Wild-type	P	S	R	Y	L
1	T	P	Y	L	M
2, 4	Y	G	Y	L	M
3*	Y	R	Y	R	M
5, 12	T	H	Y	R	G
6	T	H	Y	R	M
7*	Y	K	Y	R	M
8, 9	T	K	Y	R	G
10	Y	K	Y	R	.

*Variant also contained Met226Ile.

Table 7: Library G Variants

	Position in HRG-β1					
Variant No.	211	212	213	214	215	216
Wild-type	K	C	P	N	E	F
1, 5, 6, 10, 12	R	.	S	L	.	.
2	R	.	S	E	.	.
3	.	.	.	K	.	M
4	R	.	T	V	.	Y
7, 8	R	.	T	V	.	Y
9	.	.	N	S	.	.
11	R	.	K	K	.	.

Example 2 - HER2/HER3 expression in embryonic rat lung

Rat lungs were microdissected from rat embryos on embryologic day (E) 16, 18, 20, and post-natal (P) days 7, 14, and adult. Isolated lung tissue was homogenized in a standard protease inhibitor buffer, and equal protein amounts subjected to SDS-PAGE (4-20%), blotted to nitrocellulose and identified with specific antibodies (HER2, HER3, HER4-Santa Cruz Biological, San Jose, CA. and HRG, 3G11, Genentech, Inc.) using chemiluminescent techniques.

Analysis of the blot indicates that HER2 is expressed at high levels throughout development in utero, appears to peak on E18 and declines after birth to lower adult levels. HER3 expression peaks at E18,

containing overhangs. The fragment was inserted into phagemid display vector pam-g3 by restriction digest-ligation at the same sites to generate construct pHRG2-g3 (177-244). pam-g3 was a derivative of phGHam-g3, which was designed for phage display of human growth hormone (hGH) and was described in Lowman et al., *Biochemistry* 30:10832-10838 (1991). pam-g3 was produced by removing the hGH gene present in phGHam-g3 and replacing this gene with a stuffer fragment, which provides space for cleavage at the restriction sites used for cloning. The HRG-β1 fragment was attached to residue 247 of pIII.

The HRG-β1 EGF-like domain expressed from the above-described construct is designated by removing the "p" and the "-g3" that appear in the name of the construct. Thus, the HRG-β1 EGF-like domain expressed from the pHRG2-g3 construct is designated "HRG2."

The domain was displayed monovalently on phage as a pIII fusion protein, as described by Bass et al., *Proteins* 8:309-314 (1990).

Similarly, variants HRG-$\beta1_{147-227}$, HRG-$\beta1_{147-244}$, and HRG-$\beta1_{177-227}$ were prepared and expressed as described above.

Example 6 - rHRG$\beta1_{177-244}$ causes accelerated lung development

Exogenous rHRG$\beta1_{177-244}$ caused accelerated lung development *in vitro*. To determine if the expressed HER2/HER3 receptors were functional and the role of HRG stimulation during lung development *in vitro*, rHRG$\beta1_{177-244}$ was added to the *in vitro* culture at 10 nM. Tissue was harvested at D5, snap frozen, and 5 micron sections were cut for analysis.

The morphology, in comparison to the untreated control specimens was grossly different. There was marked proliferation of the epithelium. Air spaces which are typically lined with a single cell layer now had a 2-3 cell thickness. The changes were dose dependent with more epithelial cell response with higher concentrations. HER2 and HER3 were still identifiable in the epithelium only.

Example 7 - Human lung differentiation

Differentiation of human lung epithelial cells occurs after HRG treatment. Differentiation was measured by Surfactant Protein A (SPA) production. All sections were stained for SPA. Human lung explant stained for SPA with stain localizing in epithelial cells of the prealveolar ducts. Human lung explant exposed to 10 nM HRG showed an effect on SPA production. As a differentiation control, a lung explant was exposed to 1mM dibutyryl cAMP. A negative control was also run.

17. The method of Claim 16, wherein the activating ligand is a heregulin (HRG) polypeptide, HRG variant, HRG agonist antibody or fragment thereof capable of binding to the HER2, HER3 and/or HER4 receptor.

18. A method of treating respiratory distress or emphysema, comprising administering to a patient in need thereof an effective amount of an isolated HER2, HER3 and/or HER4 activating ligand.

19. The method of Claim 18, wherein the activating ligand is a heregulin (HRG) polypeptide, HRG variant, HRG agonist antibody or fragment thereof capable of binding to the HER2, HER3 and/or HER4 receptor.

20. A method, comprising the steps of:

(a) obtaining a normal epithelial cell sample from a mammal;

(b) contacting the sample with a ligand which activates HER2, HER3, HER4 or a combination thereof to induce growth and/or proliferation of epithelial cells in the sample and to obtain an expanded sample; and

(c) re-introducing the expanded sample into the mammal.

```
GG  GCG CGA GCG CCT CAG CGC GGC CGC TCG CTC TCC CCC 38
    Ala Arg Ala Pro Gln Arg Gly Arg Ser Leu Ser Pro
      1               5                  10

TCG AGG GAC AAA CTT TTC CCA AAC CCG ATC CGA GCC CTT 77
Ser Arg Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu
         15                  20                  25

GGA CCA AAC TCG CCT GCG CCG AGA GCC GTC CGC GTA GAG 116
Gly Pro Asn Ser Pro Ala Pro Arg Ala Val Arg Val Glu
                30                  35

CGC TCC GTC TCC GGC GAG ATG TCC GAG CGC AAA GAA GGC 155
Arg Ser Val Ser Gly Glu Met Ser Glu Arg Lys Glu Gly
     40                  45                  50

AGA GGC AAA GGG AAG GGC AAG AAG AAG GAG CGA GGC TCC 194
Arg Gly Lys Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser
            55                  60

GGC AAG AAG CCG GAG TCC GCG GCG GGC AGC CAG AGC CCA 233
Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro
 65                  70                  75

GCC TTG CCT CCC CGA TTG AAA GAG ATG AAA AGC CAG GAA 272
Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
         80                  85                  90

TCG GCT GCA GGT TCC AAA CTA GTC CTT CGG TGT GAA ACC 311
Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr
                95                 100

AGT TCT GAA TAC TCC TCT CTC AGA TTC AAG TGG TTC AAG 350
Ser Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys
    105                 110                 115

AAT GGG AAT GAA TTG AAT CGA AAA AAC AAA CCA CAA AAT 389
Asn Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn
            120                 125

ATC AAG ATA CAA AAA AAG CCA GGG AAG TCA GAA CTT CGC 428
Ile Lys Ile Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg
130                 135                 140

ATT AAC AAA GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG 467
Ile Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met
        145                 150                 155

TGC AAA GTG ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT 506
Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser
            160                 165
```

FIG._1A

```
GCC AAT ATC ACC ATC GTG GAA TCA AAC GAG ATC ATC ACT 545
Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile Ile Thr
    170                 175                 180

GGT ATG CCA GCC TCA ACT GAA GGA GCA TAT GTG TCT TCA 584
Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
            185                 190

GAG TCT CCC ATT AGA ATA TCA GTA TCC ACA GAA GGA GCA 623
Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala
195                 200                 205

AAT ACT TCT TCA TCT ACA TCT ACA TCC ACC ACT GGG ACA 662
Asn Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr
        210                 215                 220

AGC CAT CTT GTA AAA TGT GCG GAG AAG GAG AAA ACT TTC 701
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe
                225                 230

TGT GTG AAT GGA GGG GAG TGC TTC ATG GTG AAA GAC CTT 740
Cys Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu
    235                 240                 245

TCA AAC CCC TCG AGA TAC TTG TGC AAG TGC CAA CCT GGA 779
Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys Gln Pro Gly
            250                 255

TTC ACT GGA GCA AGA TGT ACT GAG AAT GTG CCC ATG AAA 818
Phe Thr Gly Ala Arg Cys Thr Glu Asn Val Pro Met Lys
260                 265                 270

GTC CAA AAC CAA GAA AAG GCG GAG GAG CTG TAC CAG AAG 857
Val Gln Asn Gln Glu Lys Ala Glu Glu Leu Tyr Gln Lys
        275                 280                 285

AGA GTG CTG ACC ATA ACC GGC ATC TGC ATC GCC CTC CTT 896
Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu
                290                 295

GTG GTC GGC ATC ATG TGT GTG GTG GCC TAC TGC AAA ACC 935
Val Val Gly Ile Met Cys Val Val Ala Tyr Cys Lys Thr
    300                 305                 310

AAG AAA CAG CGG AAA AAG CTG CAT GAC CGT CTT CGG CAG 974
Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln
            315                 320

AGC CTT CGG TCT GAA CGA AAC AAT ATG ATG AAC ATT GCC 1013
Ser Leu Arg Ser Glu Arg Asn Asn Met Met Asn Ile Ala
325                 330                 335
```

FIG._1B

```
AAT GGG CCT CAC CAT CCT AAC CCA CCC CCC GAG AAT GTC 1052
Asn Gly Pro His His Pro Asn Pro Pro Pro Glu Asn Val
        340                 345                 350

CAG CTG GTG AAT CAA TAC GTA TCT AAA AAC GTC ATC TCC 1091
Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile Ser
                355                 360

AGT GAG CAT ATT GTT GAG AGA GAA GCA GAG ACA TCC TTT 1130
Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser Phe
    365                 370                 375

TCC ACC AGT CAC TAT ACT TCC ACA GCC CAT CAC TCC ACT 1169
Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr
            380                 385

ACT GTC ACC CAG ACT CCT AGC CAC AGC TGG AGC AAC GGA 1208
Thr Val Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly
390                 395                 400

CAC ACT GAA AGC ATC CTT TCC GAA AGC CAC TCT GTA ATC 1247
His Thr Glu Ser Ile Leu Ser Glu Ser His Ser Val Ile
        405                 410                 415

GTG ATG TCA TCC GTA GAA AAC AGT AGG CAC AGC AGC CCA 1286
Val Met Ser Ser Val Glu Asn Ser Arg His Ser Ser Pro
                420                 425

ACT GGG GGC CCA AGA GGA CGT CTT AAT GGC ACA GGA GGC 1325
Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly Thr Gly Gly
    430                 435                 440

CCT CGT GAA TGT AAC AGC TTC CTC AGG CAT GCC AGA GAA 1364
Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala Arg Glu
            445                 450

ACC CCT GAT TCC TAC CGA GAC TCT CCT CAT AGT GAA AGG 1403
Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg
455                 460                 465

TAT GTG TCA GCC ATG ACC ACC CCG GCT CGT ATG TCA CCT 1442
Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro
        470                 475                 480

GTA GAT TTC CAC ACG CCA AGC TCC CCC AAA TCG CCC CCT 1481
Val Asp Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro
                485                 490

TCG GAA ATG TCT CCA CCC GTG TCC AGC ATG ACG GTG TCC 1520
Ser Glu Met S r Pro Pro Val Ser Ser M t Thr Val Ser
    495                 500                 505
```

FIG._1C

```
ATG CCT TCC ATG GCG GTC AGC CCC TTC ATG GAA GAA GAG 1559
Met Pro Ser Met Ala Val Ser Pro Phe Met Glu Glu Glu
            510                 515

AGA CCT CTA CTT CTC GTG ACA CCA CCA AGG CTG CGG GAG 1598
Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu Arg Glu
520                 525                 530

AAG AAG TTT GAC CAT CAC CCT CAG CAG TTC AGC TCC TTC 1637
Lys Lys Phe Asp His His Pro Gln Gln Phe Ser Ser Phe
        535                 540                 545

CAC CAC AAC CCC GCG CAT GAC AGT AAC AGC CTC CCT GCT 1676
His His Asn Pro Ala His Asp Ser Asn Ser Leu Pro Ala
                550                 555

AGC CCC TTG AGG ATA GTG GAG GAT GAG GAG TAT GAA ACG 1715
Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu Thr
    560                 565                 570

ACC CAA GAG TAC GAG CCA GCC CAA GAG CCT GTT AAG AAA 1754
Thr Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys
            575                 580

CTC GCC AAT AGC CGG CGG GCC AAA AGA ACC AAG CCC AAT 1793
Leu Ala Asn Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn
585                 590                 595

GGC CAC ATT GCT AAC AGA TTG GAA GTG GAC AGC AAC ACA 1832
Gly His Ile Ala Asn Arg Leu Glu Val Asp Ser Asn Thr
        600                 605                 610

AGC TCC CAG AGC AGT AAC TCA GAG AGT GAA ACA GAA GAT 1871
Ser Ser Gln Ser Ser Asn Ser Glu Ser Glu Thr Glu Asp
                615                 620

GAA AGA GTA GGT GAA GAT ACG CCT TTC CTG GGC ATA CAG 1910
Glu Arg Val Gly Glu Asp Thr Pro Phe Leu Gly Ile Gln
    625                 630                 635

AAC CCC CTG GCA GCC AGT CTT GAG GCA ACA CCT GCC TTC 1949
Asn Pro Leu Ala Ala Ser Leu Glu Ala Thr Pro Ala Phe
            640                 645

CGC CTG GCT GAC AGC AGG ACT AAC CCA GCA GGC CGC TTC 1988
Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala Gly Arg Phe
650                 655                 660

TCG ACA CAG GAA GAA ATC CAG G 2010
Ser Thr Gln Glu Glu Ile Gln
        665                 669
```

FIG._1D

```
GG  GAC AAA CTT TTC CCA AAC CCG ATC CGA GCC CTT GGA 38
    Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu Gly
      1               5                  10

CCA AAC TCG CCT GCG CCG AGA GCC GTC CGC GTA GAG CGC 77
Pro Asn Ser Pro Ala Pro Arg Ala Val Arg Val Glu Arg
         15                  20                  25

TCC GTC TCC GGC GAG ATG TCC GAG CGC AAA GAA GGC AGA 116
Ser Val Ser Gly Glu Met Ser Glu Arg Lys Glu Gly Arg
                30                  35

GGC AAA GGG AAG GGC AAG AAG AAG GAG CGA GGC TCC GGC 155
Gly Lys Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser Gly
    40                  45                  50

AAG AAG CCG GAG TCC GCG GCG GGC AGC CAG AGC CCA GCC 194
Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro Ala
            55                  60

TTG CCT CCC CAA TTG AAA GAG ATG AAA AGC CAG GAA TCG 233
Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser
 65                  70                  75

GCT GCA GGT TCC AAA CTA GTC CTT CGG TGT GAA ACC AGT 272
Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
         80                  85                  90

TCT GAA TAC TCC TCT CTC AGA TTC AAG TGG TTC AAG AAT 311
Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn
                95                 100

GGG AAT GAA TTG AAT CGA AAA AAC AAA CCA CAA AAT ATC 350
Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile
    105                 110                 115

AAG ATA CAA AAA AAG CCA GGG AAG TCA GAA CTT CGC ATT 389
Lys Ile Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile
            120                 125

AAC AAA GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG TGC 428
Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys
130                 135                 140

AAA GTG ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT GCC 467
Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala
        145                 150                 155

AAT ATC ACC ATC GTG GAA TCA AAC GAG ATC ATC ACT GGT 506
Asn Ile Thr Ile Val Glu Ser Asn Glu Il  Ile Thr Gly
                160                 165
```

FIG._2A

```
ATG CCA GCC TCA ACT GAA GGA GCA TAT GTG TCT TCA GAG 545
Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu
    170                 175                 180

TCT CCC ATT AGA ATA TCA GTA TCC ACA GAA GGA GCA AAT 584
Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn
            185                 190

ACT TCT TCA TCT ACA TCT ACA TCC ACC ACT GGG ACA AGC 623
Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser
195                 200                 205

CAT CTT GTA AAA TGT GCG GAG AAG GAG AAA ACT TTC TGT 662
His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys
        210                 215                 220

GTG AAT GGA GGG GAG TGC TTC ATG GTG AAA GAC CTT TCA 701
Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser
            225                 230

AAC CCC TCG AGA TAC TTG TGC AAG TGC CCA AAT GAG TTT 740
Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe
    235                 240                 245

ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC 779
Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe
            250                 255

TAC AAG CAT CTT GGG ATT GAA TTT ATG GAG GCG GAG GAG 818
Tyr Lys His Leu Gly Ile Glu Phe Met Glu Ala Glu Glu
260                 265                 270

CTG TAC CAG AAG AGA GTG CTG ACC ATA ACC GGC ATC TGC 857
Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys
        275                 280                 285

ATC GCC CTC CTT GTG GTC GGC ATC ATG TGT GTG GTG GCC 896
Ile Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala
            290                 295

TAC TGC AAA ACC AAG AAA CAG CGG AAA AAG CTG CAT GAC 935
Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp
    300                 305                 310

CGT CTT CGG CAG AGC CTT CGG TCT GAA CGA AAC AAT ATG 974
Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn Met
            315                 320

ATG AAC ATT GCC AAT GGG CCT CAC CAT CCT AAC CCA CCC 1013
Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro
325                 330                 335
```

FIG._2B

```
CCC GAG AAT GTC CAG CTG GTG AAT CAA TAC GTA TCT AAA 1052
Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys
        340                 345                 350

AAC GTC ATC TCC AGT GAG CAT ATT GTT GAG AGA GAA GCA 1091
Asn Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala
                355                 360

GAG ACA TCC TTT TCC ACC AGT CAC TAT ACT TCC ACA GCC 1130
Glu Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala
    365                 370                 375

CAT CAC TCC ACT ACT GTC ACC CAG ACT CCT AGC CAC AGC 1169
His His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser
            380                 385

TGG AGC AAC GGA CAC ACT GAA AGC ATC CTT TCC GAA AGC 1208
Trp Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser
390                 395                 400

CAC TCT GTA ATC GTG ATG TCA TCC GTA GAA AAC AGT AGG 1247
His Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg
        405                 410                 415

CAC AGC AGC CCA ACT GGG GGC CCA AGA GGA CGT CTT AAT 1286
His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
                420                 425

GGC ACA GGA GGC CCT CGT GAA TGT AAC AGC TTC CTC AGG 1325
Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg
    430                 435                 440

CAT GCC AGA GAA ACC CCT GAT TCC TAC CGA GAC TCT CCT 1364
His Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro
            445                 450

CAT AGT GAA AGG TAT GTG TCA GCC ATG ACC ACC CCG GCT 1403
His Ser Glu Arg Tyr Val Ser Ala Met Thr Thr Pro Ala
455                 460                 465

CGT ATG TCA CCT GTA GAT TTC CAC ACG CCA AGC TCC CCC 1442
Arg Met Ser Pro Val Asp Phe His Thr Pro Ser Ser Pro
        470                 475                 480

AAA TCG CCC CCT TCG GAA ATG TCT CCA CCC GTG TCC AGC 1481
Lys Ser Pro Pro Ser Glu Met Ser Pro Pro Val Ser Ser
                485                 490

ATG ACG GTG TCC ATG CCT TCC ATG GCG GTC AGC CCC TTC 1520
Met Thr Val Ser Met Pro Ser Met Ala Val S r Pro Phe
    495                 500                 505
```

FIG._2C

```
ATG GAA GAA GAG AGA CCT CTA CTT CTC GTG ACA CCA CCA 1559
Met Glu Glu Glu Arg Pro Leu Leu Leu Val Thr Pro Pro
               510                 515

AGG CTG CGG GAG AAG AAG TTT GAC CAT CAC CCT CAG CAG 1598
Arg Leu Arg Glu Lys Lys Phe Asp His His Pro Gln Gln
520                 525                 530

TTC AGC TCC TTC CAC CAC AAC CCC GCG CAT GAC AGT AAC 1637
Phe Ser Ser Phe His His Asn Pro Ala His Asp Ser Asn
        535                 540                 545

AGC CTC CCT GCT AGC CCC TTG AGG ATA GTG GAG GAT GAG 1676
Ser Leu Pro Ala Ser Pro Leu Arg Ile Val Glu Asp Glu
                550                 555

GAG TAT GAA ACG ACC CAA GAG TAC GAG CCA GCC CAA GAG 1715
Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln Glu
    560                 565                 570

CCT GTT AAG AAA CTC GCC AAT AGC CGG CGG GCC AAA AGA 1754
Pro Val Lys Lys Leu Ala Asn Ser Arg Arg Ala Lys Arg
               575                 580

ACC AAG CCC AAT GGC CAC ATT GCT AAC AGA TTG GAA GTG 1793
Thr Lys Pro Asn Gly His Ile Ala Asn Arg Leu Glu Val
585                 590                 595

GAC AGC AAC ACA AGC TCC CAG AGC AGT AAC TCA GAG AGT 1832
Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn Ser Glu Ser
        600                 605                 610
```

FIG._2D

```
GAA ACA GAA GAT GAA AGA GTA GGT GAA GAT ACG CCT TTC 1871
Glu Thr Glu Asp Glu Arg Val Gly Glu Asp Thr Pro Phe
                615                 620

CTG GGC ATA CAG AAC CCC CTG GCA GCC AGT CTT GAG GCA 1910
Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu Ala
    625                 630                 635

ACA CCT GCC TTC CGC CTG GCT GAC AGC AGG ACT AAC CCA 1949
Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro
            640                 645

GCA GGC CGC TTC TCG ACA CAG GAA GAA ATC CAG GCC AGG 1988
Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile Gln Ala Arg
650                 655                 660

CTG TCT AGT GTA ATT GCT AAC CAA GAC CCT ATT GCT GTA TA 2029
Leu Ser Ser Val Ile Ala Asn Gln Asp Pro Ile Ala Val
        665                 670                 675

A AACCTAAATA AACACATAGA TTCACCTGTA AAACTTTATT              2070

TTATATAATA AAGTATTCCA CCTTAAATTA AACAATTTAT TTTATTTTAG    2120

CAGTTCTGCA AATAGAAAAC AGGAAAAAAA CTTTTATAAA TTAAATATAT    2170

GTATGTAAAA ATGAAAAAAA AAAAAAAAA                           2199
```

FIG._2E

```
GTGGCTGCGG GGCAATTGAA AAAGAGCCGG CGAGGAGTTC CCCGAAACTT 50

GTTGGAACTC CGGGCTCGCG CGGAGGCCAG GAGCTGAGCG GCGGCGGCTG 100

CCGGACGATG GGAGCGTGAG CAGGACGGTG ATAACCTCTC CCCGATCGGG 150

TTGCGAGGGC GCCGGGCAGA GGCCAGGACG CGAGCCGCCA GCGGCGGGAC 200

CCATCGACGA CTTCCCGGGG CGACAGGAGC AGCCCCGAGA GCCAGGGCGA 250

GCGCCCGTTC CAGGTGGCCG GACCGCCCGC CGCGTCCGCG CCGCGCTCCC 300

TGCAGGCAAC GGGAGACGCC CCCGCGCAGC GCGAGCGCCT CAGCGCGGCC 350

GCTCGCTCTC CCCATCGAGG GACAAACTTT TCCCAAACCC GATCCGAGCC 400

CTTGGACCAA ACTCGCCTGC GCCGAGAGCC GTCCGCGTAG AGCGCTCCGT 450

CTCCGGCGAG  ATG TCC GAG CGC AAA GAA GGC AGA GGC AAA 490
            Met Ser Glu Arg Lys Glu Gly Arg Gly Lys
              1               5                  10

GGG AAG GGC AAG AAG AAG GAG CGA GGC TCC GGC AAG AAG 529
Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser Gly Lys Lys
                  15                  20

CCG GAG TCC GCG GCG GGC AGC CAG AGC CCA GCC TTG CCT 568
Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro Ala Leu Pro
     25                  30                  35

CCC CAA TTG AAA GAG ATG AAA AGC CAG GAA TCG GCT GCA 607
Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser Ala Ala
             40                  45

GGT TCC AAA CTA GTC CTT CGG TGT GAA ACC AGT TCT GAA 646
Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
 50                  55                  60

TAC TCC TCT CTC AGA TTC AAG TGG TTC AAG AAT GGG AAT 685
Tyr Ser Ser Leu Arg Ph  Lys Trp Phe Lys Asn Gly Asn
         65                  70                  75

GAA TTG AAT CGA AAA AAC AAA CCA CAA AAT ATC AAG ATA 724
Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile
                 80                  85
```

FIG._3A

```
CAA AAA AAG CCA GGG AAG TCA GAA CTT CGC ATT AAC AAA 763
Gln Ly  Lys Pro Gly Lys S r Glu Leu Arg Il  Asn Lys
     90                  95                 100

GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG TGC AAA GTG 802
Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val
            105                 110

ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT GCC AAT ATC 841
Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile
115                 120                 125

ACC ATC GTG GAA TCA AAC GAG ATC ATC ACT GGT ATG CCA 880
Thr Ile Val Glu Ser Asn Glu Ile Ile Thr Gly Met Pro
        130                 135                 140

GCC TCA ACT GAA GGA GCA TAT GTG TCT TCA GAG TCT CCC 919
Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu Ser Pro
                145                 150

ATT AGA ATA TCA GTA TCC ACA GAA GGA GCA AAT ACT TCT 958
Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr Ser
    155                 160                 165

TCA TCT ACA TCT ACA TCC ACC ACT GGG ACA AGC CAT CTT 997
Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu
            170                 175

GTA AAA TGT GCG GAG AAG GAG AAA ACT TTC TGT GTG AAT 1036
Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
180                 185                 190

GGA GGG GAG TGC TTC ATG GTG AAA GAC CTT TCA AAC CCC 1075
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro
        195                 200                 205

TCG AGA TAC TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT 1114
Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly
                210                 215

GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AAG 1153
Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys
    220                 225                 230

GCG GAG GAG CTG TAC CAG AAG AGA GTG CTG ACC ATA ACC 1192
Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr
            235                 240

GGC ATC TGC ATC GCC CTC CTT GTG GTC GGC ATC ATG TGT 1231
Gly Ile Cys Ile Ala Leu Leu Val Val Gly Ile Met Cys
245                 250                 255

GTG GTG GCC TAC TGC AAA ACC AAG AAA CAG CGG AAA AAG 1270
Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys
        260                 265                 270
```

FIG._3B

```
CTG CAT GAC CGT CTT CGG CAG AGC CTT CGG TCT GAA CGA 1309
Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg
                275                 280

AAC AAT ATG ATG AAC ATT GCC AAT GGG CCT CAC CAT CCT 1348
Asn Asn Met Met Asn Ile Ala Asn Gly Pro His His Pro
    285                 290                 295

AAC CCA CCC CCC GAG AAT GTC CAG CTG GTG AAT CAA TAC 1387
Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr
            300                 305

GTA TCT AAA AAC GTC ATC TCC AGT GAG CAT ATT GTT GAG 1426
Val Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu
310                 315                 320

AGA GAA GCA GAG ACA TCC TTT TCC ACC AGT CAC TAT ACT 1465
Arg Glu Ala Glu Thr Ser Phe Ser Thr Ser His Tyr Thr
        325                 330                 335

TCC ACA GCC CAT CAC TCC ACT ACT GTC ACC CAG ACT CCT 1504
Ser Thr Ala His His Ser Thr Thr Val Thr Gln Thr Pro
                340                 345

AGC CAC AGC TGG AGC AAC GGA CAC ACT GAA AGC ATC CTT 1543
Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile Leu
    350                 355                 360

TCC GAA AGC CAC TCT GTA ATC GTG ATG TCA TCC GTA GAA 1582
Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu
            365                 370

AAC AGT AGG CAC AGC AGC CCA ACT GGG GGC CCA AGA GGA 1621
Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly
375                 380                 385

CGT CTT AAT GGC ACA GGA GGC CCT CGT GAA TGT AAC AGC 1660
Arg Leu Asn Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser
        390                 395                 400

TTC CTC AGG CAT GCC AGA GAA ACC CCT GAT TCC TAC CGA 1699
Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr Arg
                405                 410

GAC TCT CCT CAT AGT GAA AGG TAT GTG TCA GCC ATG ACC 1738
Asp Ser Pro His Ser Glu Arg Tyr Val Ser Ala Met Thr
    415                 420                 425

ACC CCG GCT CGT ATG TCA CCT GTA GAT TTC CAC ACG CCA 1777
Thr Pr  Ala Arg Met Ser Pro Val Asp Phe His Thr Pr
            430                 435

AGC TCC CCC AAA TCG CCC CCT TCG GAA ATG TCT CCA CCC 1816
Ser Ser Pr  Lys Ser Pro Pro Ser Glu Met Ser Pro Pro
440                 445                 450
```

FIG._3C

```
GTG TCC AGC ATG ACG GTG TCC AAG CCT TCC ATG GCG GTC 1855
Val Ser Ser Met Thr Val Ser Lys Pro Ser Met Ala Val
        455                 460                 465

AGC CCC TTC ATG GAA GAA GAG AGA CCT CTA CTT CTC GTG 1894
Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu Val
                470                 475

ACA CCA CCA AGG CTG CGG GAG AAG AAG TTT GAC CAT CAC 1933
Thr Pro Pro Arg Leu Arg Glu Lys Lys Phe Asp His His
    480                 485                 490

CCT CAG CAG TTC AGC TCC TTC CAC CAC AAC CCC GCG CAT 1972
Pro Gln Gln Phe Ser Ser Phe His His Asn Pro Ala His
            495                 500

GAC AGT AAC AGC CTC CCT GCT AGC CCC TTG AGG ATA GTG 2011
Asp Ser Asn Ser Leu Pro Ala Ser Pro Leu Arg Ile Val
505                 510                 515

GAG GAT GAG GAG TAT GAA ACG ACC CAA GAG TAC GAG CCA 2050
Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro
        520                 525                 530

GCC CAA GAG CCT GTT AAG AAA CTC GCC AAT AGC CGG CGG 2089
Ala Gln Glu Pro Val Lys Lys Leu Ala Asn Ser Arg Arg
                535                 540

GCC AAA AGA ACC AAG CCC AAT GGC CAC ATT GCT AAC AGA 2128
Ala Lys Arg Thr Lys Pro Asn Gly His Ile Ala Asn Arg
    545                 550                 555

TTG GAA GTG GAC AGC AAC ACA AGC TCC CAG AGC AGT AAC 2167
Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn
            560                 565

TCA GAG AGT GAA ACA GAA GAT GAA AGA GTA GGT GAA GAT 2206
Ser Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp
570                 575                 580

ACG CCT TTC CTG GGC ATA CAG AAC CCC CTG GCA GCC AGT 2245
Thr Pro Phe Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser
        585                 590                 595

CTT GAG GCA ACA CCT GCC TTC CGC CTG GCT GAC AGC AGG 2284
Leu Glu Ala Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg
                600                 605

ACT AAC CCA GCA GGC CGC TTC TCG ACA CAG GAA GAA ATC 2323
Thr Asn Pro Ala Gly Arg Ph  Ser Thr Gln Glu Glu Ile
    610                 615                 620

CAG GCC AGG CTG TCT AGT GTA ATT GCT AAC CAA GAC CCT 2362
Gln Ala Arg Leu Ser Ser Val Ile Ala Asn Gln Asp Pro
            625                 630
```

FIG._3D

```
ATT GCT GTA TAAAACCTA AATAAACACA TAGATTCACC TGTAAAACTT 2410
Ile Ala Val
635     637

TATTTTATAT AATAAAGTAT TCCACCTTAA ATTAAACAAT TTATTTTATT 2460


TTAGCAGTTC TGCAAATAAA AAAAAAAAAA 2490
```

FIG._3E

```
GCGCCTGCCT CCAACCTGCG GGCGGGAGGT GGGTGGCTGC GGGGCAATTG 50

AAAAAGAGCC GGCGAGGAGT TCCCCGAAAC TTGTTGGAAC TCCGGGCTCG 100

CGCGGAGGCC AGGAGCTGAG CGGCGGCGGC TGCCGGACGA TGGGAGCGTG 150

AGCAGGACGG TGATAACCTC TCCCCGATCG GGTTGCGAGG GCGCCGGGCA 200

GAGGCCAGGA CGCGAGCCGC CAGCGGCGGG ACCCATCGAC GACTTCCCGG 250

GGCGACAGGA GCAGCCCCGA GAGCCAGGGC GAGCGCCCGT TCCAGGTGGC 300

CGGACCGCCC GCCGCGTCCG CGCCGCGCTC CCTGCAGGCA ACGGGAGACG 350

CCCCCGCGCA GCGCGAGCGC CTCAGCGCGG CCGCTCGCTC TCCCCATCGA 400

GGGACAAACT TTTCCCAAAC CCGATCCGAG CCCTTGGACC AAACTCGCCT 450

GCGCCGAGAG CCGTCCGCGT AGAGCGCTCC GTCTCCGGCG AG  ATG 495
                                                Met
                                                  1

TCC GAG CGC AAA GAA GGC AGA GGC AAA GGG AAG GGC AAG 534
Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys
          5                  10

AAG AAG GAG CGA GGC TCC GGC AAG AAG CCG GAG TCC GCG 573
Lys Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala
 15                  20                  25

GCG GGC AGC CAG AGC CCA GCC TTG CCT CCC CAA TTG AAA 612
Ala Gly Ser Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys
         30                  35                  40

GAG ATG AAA AGC CAG GAA TCG GCT GCA GGT TCC AAA CTA 651
Glu Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu
                45                  50

GTC CTT CGG TGT GAA ACC AGT TCT GAA TAC TCC TCT CTC 690
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
     55                  60                  65

AGA TTC AAG TGG TTC AAG AAT GGG AAT GAA TTG AAT CGA 729
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg
          70                  75
```

FIG._4A

```
AAA AAC AAA CCA CAA AAT ATC AAG ATA CAA AAA AAG CCA 768
Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro
 80                  85                  90

GGG AAG TCA GAA CTT CGC ATT AAC AAA GCA TCA CTG GCT 807
Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
         95                 100                 105

GAT TCT GGA GAG TAT ATG TGC AAA GTG ATC AGC AAA TTA 846
Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
                110                 115

GGA AAT GAC AGT GCC TCT GCC AAT ATC ACC ATC GTG GAA 885
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu
    120                 125                 130

TCA AAC GAG ATC ATC ACT GGT ATG CCA GCC TCA ACT GAA 924
Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
            135                 140

GGA GCA TAT GTG TCT TCA GAG TCT CCC ATT AGA ATA TCA 963
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser
145                 150                 155

GTA TCC ACA GAA GGA GCA AAT ACT TCT TCA TCT ACA TCT 1002
Val Ser Thr Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser
        160                 165                 170

ACA TCC ACC ACT GGG ACA AGC CAT CTT GTA AAA TGT GCG 1041
Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala
                175                 180

GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC 1080
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
    185                 190                 195

TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG 1119
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
            200                 205

TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA 1158
Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln
210                 215                 220

AAC TAC GTA ATG GCC AGC TTC TAC AGT ACG TCC ACT CCC 1197
Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro
        225                 230                 235

TTT CTG TCT CTG CCT GAA TAGGA GCATGCTCAG TTGGTGCTGC 1240
Phe Leu Ser Leu Pro Glu
                240 241

TTTCTTGTTG CTGCATCTCC CCTCAGATTC CACCTAGAGC TAGATGTGTC 1290
```

FIG._4B

TTACCAGATC TAATATTGAC TGCCTCTGCC TGTCGCATGA GAACATTAAC 1340

AAAAGCAATT GTATTACTTC CTCTGTTCGC GACTAGTTGG CTCTGAGATA 1390

CTAATAGGTG TGTGAGGCTC CGGATGTTTC TGGAATTGAT ATTGAATGAT 1440

GTGATACAAA TTGATAGTCA ATATCAAGCA GTGAAATATG ATAATAAAGG 1490

CATTTCAAAG TCTCACTTTT ATTGATAAAA TAAAAATCAT TCTACTGAAC 1540

AGTCCATCTT CTTTATACAA TGACCACATC CTGAAAAGGG TGTTGCTAAG 1590

CTGTAACCGA TATGCACTTG AAATGATGGT AAGTTAATTT TGATTCAGAA 1640

TGTGTTATTT GTCACAAATA AACATAATAA AAGGAGTTCA GATGTTTTTC 1690

TTCATTAACC AAAAAAAAAA AAAAA 1715

FIG._4C

```
GAGGCGCCTG CCTCCAACCT GCGGGCGGGA GGTGGGTGGC TGCGGGGCAA 50

TTGAAAAAGA GCCGGCGAGG AGTTCCCCGA AACTTGTTGG AACTCCGGGC 100

TCGCGCGGAG GCCAGGAGCT GAGCGGCGGC GGCTGCCGGA CGATGGGAGC 150

GTGAGCAGGA CGGTGATAAC CTCTCCCCGA TCGGGTTGCG AGGGCGCCGG 200

GCAGAGGCCA GGACGCGAGC CGCCAGCGGC GGGACCCATC GACGACTTCC 250

CGGGGCGACA GGAGCAGCCC CGAGAGCCAG GGCGAGCGCC CGTTCCAGGT 300

GGCCGGACCG CCCGCCGCGT CCGCGCCGCG CTCCCTGCAG GCAACGGGAG 350

ACGCCCCCGC GCAGCGCGAG CGCCTCAGCG CGGCCGCTCG CTCTCCCCAT 400

CGAGGGACAA ACTTTTCCCA AACCCGATCC GAGCCCTTGG ACCAAACTCG 450

CCTGCGCCGA GAGCCGTCCG CGTAGAGCGC TCCGTCTCCG GCGAG   AT 497
                                                    Met
                                                     1

G TCC GAG CGC AAA GAA GGC AGA GGC AAA GGG AAG GGC AAG 537
  Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys
                5                  10

AAG AAG GAG CGA GGC TCC GGC AAG AAG CCG GAG TCC GCG 576
Lys Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala
 15                 20                  25

GCG GGC AGC CAG AGC CCA GCC TTG CCT CCC CAA TTG AAA 615
Ala Gly Ser Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys
        30                  35                  40

GAG ATG AAA AGC CAG GAA TCG GCT GCA GGT TCC AAA CTA 654
Glu Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu
               45                  50

GTC CTT CGG TGT GAA ACC AGT TCT GAA TAC TCC TCT CTC 693
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
     55                  60                  65

AGA TTC AAG TGG TTC AAG AAT GGG AAT GAA TTG AAT CGA 732
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg
            70                  75
```

FIG._5A

```
AAA AAC AAA CCA CAA AAT ATC AAG ATA CAA AAA AAG CCA 771
Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro
 80                  85                  90

GGG AAG TCA GAA CTT CGC ATT AAC AAA GCA TCA CTG GCT 810
Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
          95                 100                 105

GAT TCT GGA GAG TAT ATG TGC AAA GTG ATC AGC AAA TTA 849
Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
                    110                 115

GGA AAT GAC AGT GCC TCT GCC AAT ATC ACC ATC GTG GAA 888
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu
    120                 125                 130

TCA AAC GAG ATC ATC ACT GGT ATG CCA GCC TCA ACT GAA 927
Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
              135                 140

GGA GCA TAT GTG TCT TCA GAG TCT CCC ATT AGA ATA TCA 966
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser
145                 150                 155

GTA TCC ACA GAA GGA GCA AAT ACT TCT TCA TCT ACA TCT 1005
Val Ser Thr Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser
          160                 165                 170

ACA TCC ACC ACT GGG ACA AGC CAT CTT GTA AAA TGT GCG 1044
Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala
                    175                 180

GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC 1083
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
    185                 190                 195

TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG 1122
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
              200                 205

TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA 1161
Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln
210                 215                 220

AAC TAC GTA ATG GCC AGC TTC TAC AAG GCG GAG GAG CTG 1200
Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu
          225                 230                 235

TAC CAG AAG AGA GTG CTG ACC ATA ACC GGC ATC TGC ATC 1239
Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Il  Cys Ile
                    240                 245

GCC CTC CTT GTG GTC GGC ATC ATG TGT GTG GTG GCC TAC 1278
Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr
    250                 255                 260
```

FIG._5B

```
TGC AAA ACC AAG AAA CAG CGG AAA AAG CTG CAT GAC CGT 1317
Cys Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg
            265                 270

CTT CGG CAG AGC CTT CGG TCT GAA CGA AAC AAT ATG ATG 1356
Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn Met Met
275                 280                 285

AAC ATT GCC AAT GGG CCT CAC CAT CCT AAC CCA CCC CCC 1395
Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
        290                 295                 300

GAG AAT GTC CAG CTG GTG AAT CAA TAC GTA TCT AAA AAC 1434
Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn
                305                 310

GTC ATC TCC AGT GAG CAT ATT GTT GAG AGA GAA GCA GAG 1473
Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu
    315                 320                 325

ACA TCC TTT TCC ACC AGT CAC TAT ACT TCC ACA GCC CAT 1512
Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His
            330                 335

CAC TCC ACT ACT GTC ACC CAG ACT CCT AGC CAC AGC TGG 1551
His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser Trp
340                 345                 350

AGC AAC GGA CAC ACT GAA AGC ATC CTT TCC GAA AGC CAC 1590
Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser His
        355                 360                 365

TCT GTA ATC GTG ATG TCA TCC GTA GAA AAC AGT AGG CAC 1629
Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg His
                370                 375

AGC AGC CCA ACT GGG GGC CCA AGA GGA CGT CTT AAT GGC 1668
Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly
    380                 385                 390

ACA GGA GGC CCT CGT GAA TGT AAC AGC TTC CTC AGG CAT 1707
Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His
            395                 400

GCC AGA GAA ACC CCT GAT TCC TAC CGA GAC TCT CCT CAT 1746
Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His
405                 410                 415

AGT GAA AGG TAAAA CCGAAGGCAA AGCTACTGCA GAGGAGAAAC 1790
Ser Glu Arg
        420
```

FIG._5C

TCAGTCAGAG AATCCCTGTG AGCACCTGCG GTCTCACCTC AGGAAATCTA 1840

CTCTAATCAG AATAAGGGGC GGCAGTTACC TGTTCTAGGA GTGCTCCTAG 1890

TTGATGAAGT CATCTCTTTG TTTGACGGAA CTTATTTCTT CTGAGCTTCT 1940

CTCGTCGTCC CAGTGACTGA CAGGCAACAG ACTCTTAAAG AGCTGGGATG 1990

CTTTGATGCG GAAGGTGCAG CACATGGAGT TTCCAGCTCT GGCCATGGGC 2040

TCAGACCCAC TCGGGGTCTC AGTGTCCTCA GTTGTAACAT TAGAGAGATG 2090

GCATCAATGC TTGATAAGGA CCCTTCTATA ATTCCAATTG CCAGTTATCC 2140

AAACTCTGAT TCGGTGGTCG AGCTGGCCTC GTGTTCTTAT CTGCTAACCC 2190

TGTCTTACCT TCCAGCCTCA GTTAAGTCAA ATCAAGGGCT ATGTCATTGC 2240

TGAATGTCAT GGGGGGCAAC TGCTTGCCCT CCACCCTATA GTATCTATTT 2290

TATGAAATTC CAAGAAGGGA TGAATAAATA AATCTCTTGG ATGCTGCGTC 2340

TGGCAGTCTT CACGGGTGGT TTTCAAAGCA GAAAAAAAAA AAAAAAAAAA 2390

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 2431

FIG._5D

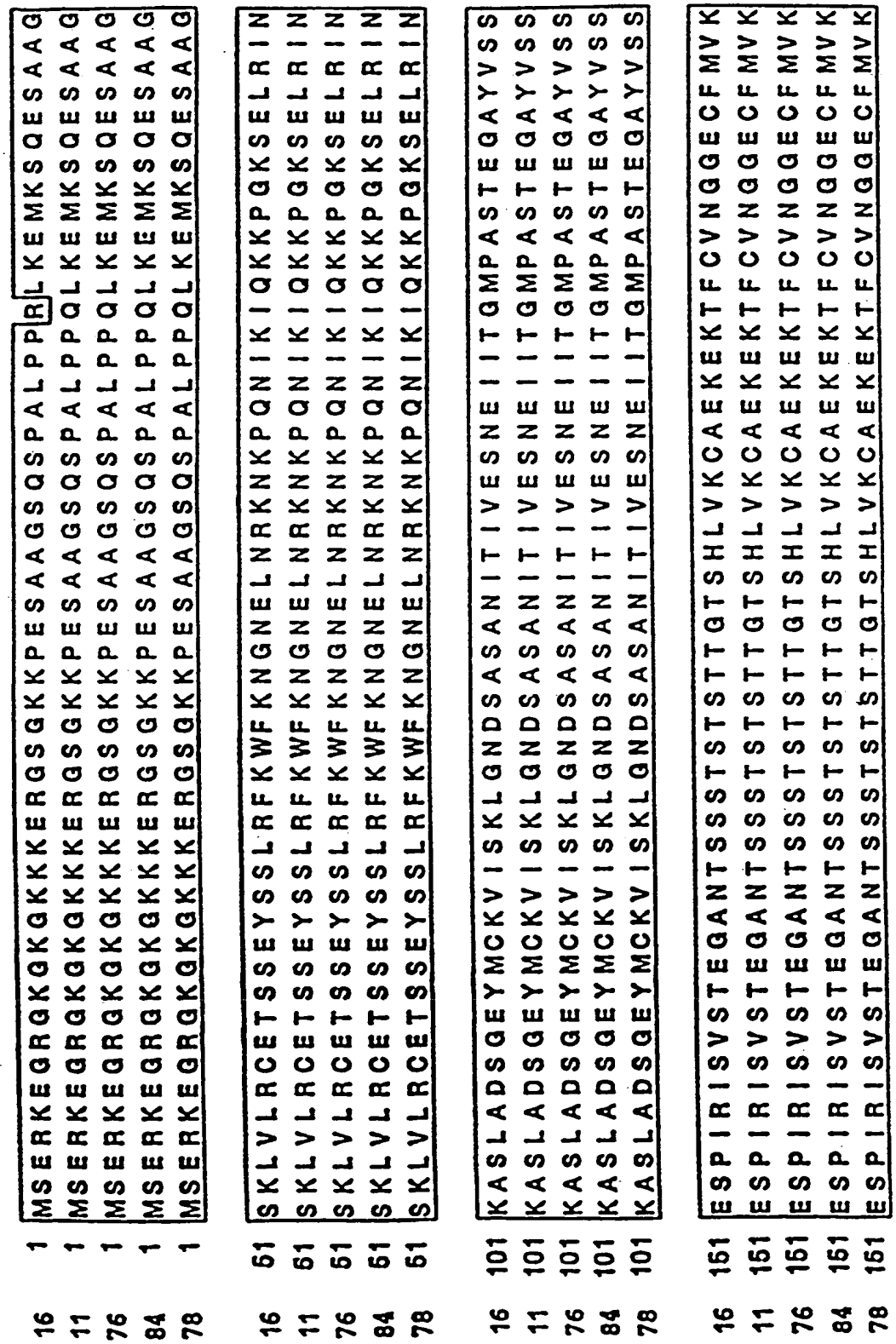
1 MSERKEGRGKGKKKERGSGKKPESAAGSQSPALPPRLKEMKSQESAAG
16 1 MSERKEGRGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG
11 1 MSERKEGRGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG
76 1 MSERKEGRGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG
84 1 MSERKEGRGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG
78 1 MSERKEGRGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG
16 51 SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
11 51 SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
76 51 SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
84 51 SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
78 51 SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
16 101 KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
11 101 KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
76 101 KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
84 101 KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
78 101 KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
16 151 ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK
11 151 ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK
76 151 ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK
84 151 ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK
78 151 ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK

FIG._6A

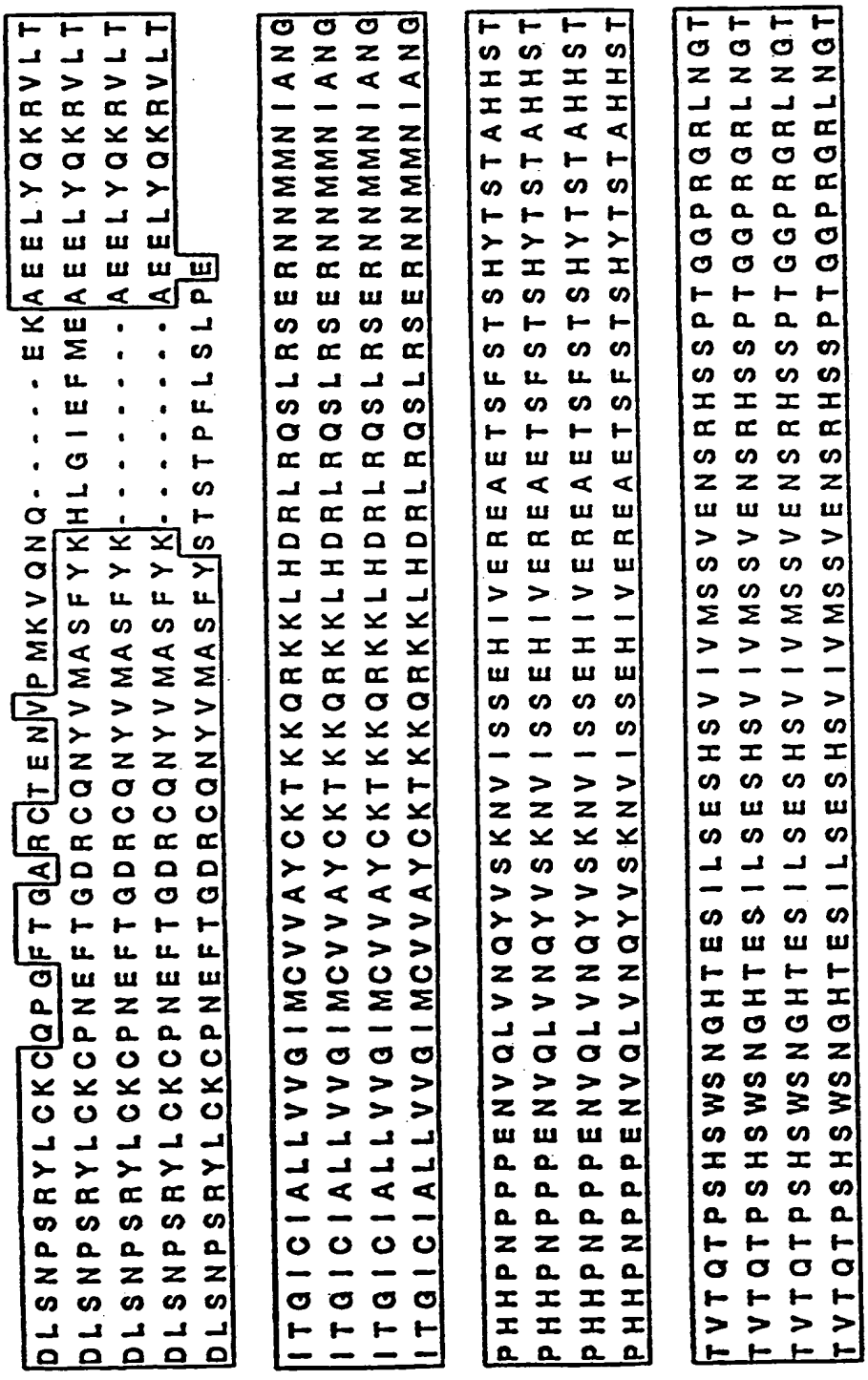
16 201 DLSNPSRYLCKCQPGFTGARCTENVPMKVQNQ- - - - - - EKAEELYQKRVLT
11 201 DLSNPSRYLCKCPNEFTGDRCQNYVMASFYKHLGIEFME AEELYQKRVLT
76 201 DLSNPSRYLCKCPNEFTGDRCQNYVMASFYK- - - - - - - - - AEELYQKRVLT
84 201 DLSNPSRYLCKCPNEFTGDRCQNYVMASFYK- - - - - - - - - AEELYQKRVLT
78 201 DLSNPSRYLCKCPNEFTGDRCQNYVMASFYSTSTPFLSLPE
16 248 ITQICIALLVVGIMCVVAYCKTKKQRKKLHDRLRQSLRSERNNMMNIANG
11 251 ITQICIALLVVGIMCVVAYCKTKKQRKKLHDRLRQSLRSERNNMMNIANG
76 243 ITQICIALLVVGIMCVVAYCKTKKQRKKLHDRLRQSLRSERNNMMNIANG
84 243 ITQICIALLVVGIMCVVAYCKTKKQRKKLHDRLRQSLRSERNNMMNIANG
16 298 PHHPNPPPENVQLVNQYVSKNVISSEHIVEREAETSFSTSHYTSTAHHST
11 301 PHHPNPPPENVQLVNQYVSKNVISSEHIVEREAETSFSTSHYTSTAHHST
76 293 PHHPNPPPENVQLVNQYVSKNVISSEHIVEREAETSFSTSHYTSTAHHST
84 293 PHHPNPPPENVQLVNQYVSKNVISSEHIVEREAETSFSTSHYTSTAHHST
16 348 TVTQTPSHSWSNGHTESILSESHSVIVMSSVENSRHSSPTGQPRQRLNGT
11 351 TVTQTPSHSWSNGHTESILSESHSVIVMSSVENSRHSSPTGQPRQRLNGT
76 343 TVTQTPSHSWSNGHTESILSESHSVIVMSSVENSRHSSPTGQPRQRLNGT
84 343 TVTQTPSHSWSNGHTESILSESHSVIVMSSVENSRHSSPTGQPRQRLNGT

FIG._6B

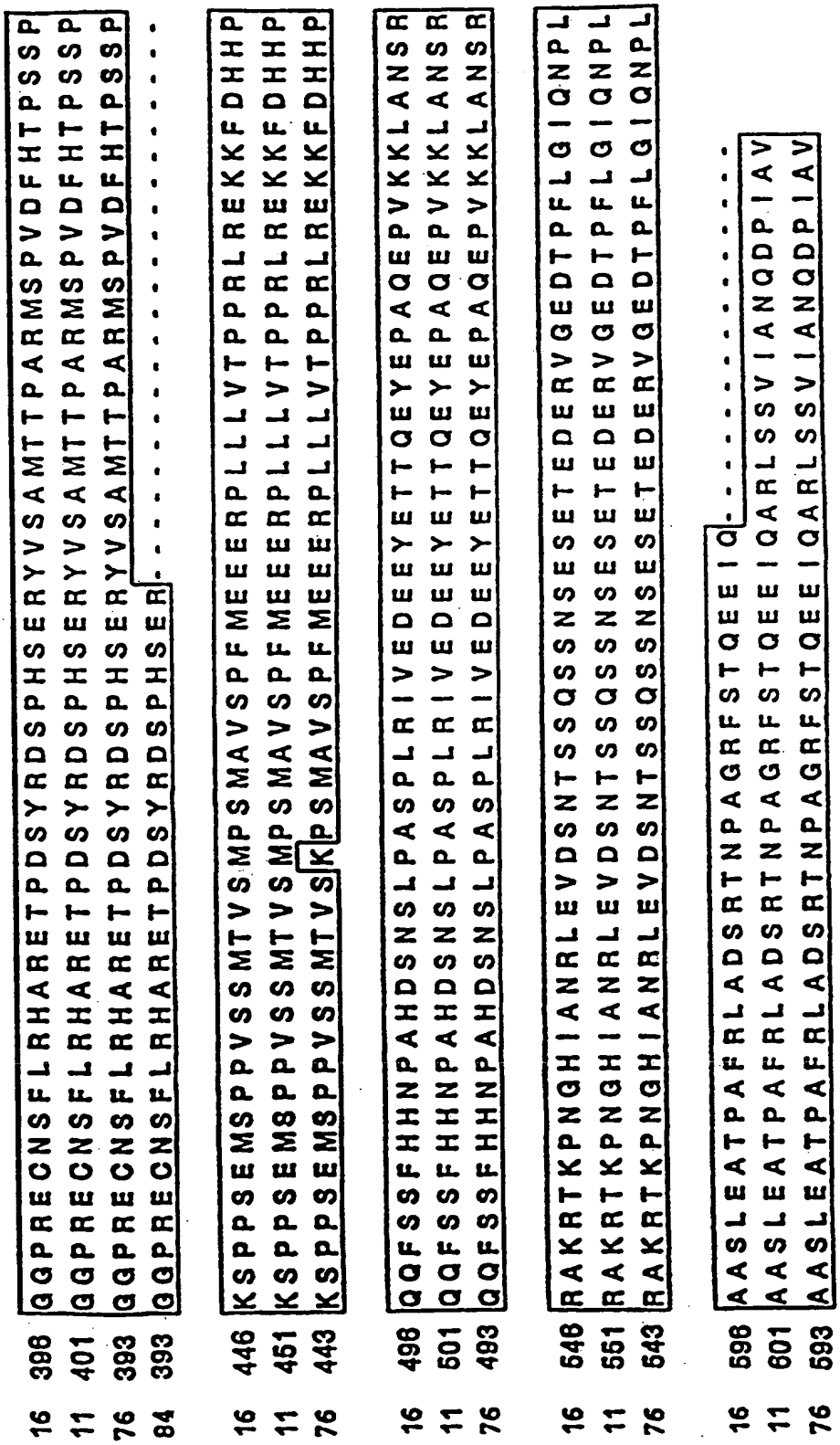
16 398 GQPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSP
11 401 GQPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSP
76 393 GQPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSP
84 393 GQPRECNSFLRHARETPDSYRDSPHSER----------
16 446 KSPPSEMSPPVSSMTVSMPSMAVSPFMEEERPLLLVTPPRLREKKFDHHP
11 451 KSPPSEMSPPVSSMTVSMPSMAVSPFMEEERPLLLVTPPRLREKKFDHHP
76 443 KSPPSEMSPPVSSMTVSKPSMAVSPFMEEERPLLLVTPPRLREKKFDHHP
16 498 QQFSSFHHNPAHDSNSLPASPLRIVEDEEYETTQEYEPAQEPVKKLANSR
11 501 QQFSSFHHNPAHDSNSLPASPLRIVEDEEYETTQEYEPAQEPVKKLANSR
76 493 QQFSSFHHNPAHDSNSLPASPLRIVEDEEYETTQEYEPAQEPVKKLANSR
16 548 RAKRTKPNGHIANRLEVDSNTSSQSSNSESETEDERVGEDTPFLQIQNPL
11 551 RAKRTKPNGHIANRLEVDSNTSSQSSNSESETEDERVGEDTPFLQIQNPL
76 543 RAKRTKPNGHIANRLEVDSNTSSQSSNSESETEDERVGEDTPFLQIQNPL
16 596 AASLEATPAFRLADSRTNPAGRFSTQEEIQ----------
11 601 AASLEATPAFRLADSRTNPAGRFSTQEEIQARLSSVIANQDPIAV
76 593 AASLEATPAFRLADSRTNPAGRFSTQEEIQARLSSVIANQDPIAV

FIG._6C

```
   1 GGGTACCATGGGTCGGTGAGCGCGTTTCCCGCCTGAGCGCAACTAGCGGC
  51 GGGTCGTGGGCACCTCCAGAAAAGATCCCGCACCATCCTCCAGGATCCAA
 101 TGGCCTTGGAGAGAGGGCTGCAGGGCCCACGGACATTGCTGACTCTTCAG
 151 AACGTGCTGACATGGAGCCAGGTAGACTGAAATTATCATGTGTCCAAATT
 201 AAAATTGCATACTTCAAGGATTATTTGAAGGACTATTCTTAGACCCTTTT
 251 AAGAAGATTTAAAGAAAAACCACTCGGCCCTGAGTGCGGCGAGGACCCTG

 301 TTTGTGGATGTGGAGGAGCGCGGGCCGGAGGCCATGGACGTGAAGGAGAG
   1                                   M  D  V  K  E  R

 351 GAAGCCTTACCGCTCGCTGACCCGGCGCCGCGACGCCGAGCGCCGCTACA
   7  K  P  Y  R  S  L  T  R  R  R  D  A  E  R  R  Y  T

 401 CCAGCTCGTCCGCGGACAGCGAGGAGGGCAAAGCCCCGCAGAAATCGTAC
  24   S  S  S  A  D  S  E  E  G  K  A  P  Q  K  S  Y

 451 AGCTCCAGCGAGACCCTGAAGGCCTACGACCAGGACGCCCGCCTAGCCTA
  40 S  S  S  E  T  L  K  A  Y  D  Q  D  A  R  L  A  Y

 501 TGGCAGCCGCGTCAAGGACATTGTGCCGCAGGAGGCCGAGGAATTCTGCC
  57  G  S  R  V  K  D  I  V  P  Q  E  A  E  E  F  C  R

 551 GCACAGGTGCCAACTTCACCCTGCGGGAGCTGGGGCTGGAAGAAGTAACG
  74   T  G  A  N  F  T  L  R  E  L  G  L  E  E  V  T

 601 CCCCCTCACGGGACCCTGTACCGGACAGACATTGGCCTCCCCCACTGCGG
  90 P  P  H  G  T  L  Y  R  T  D  I  G  L  P  H  C  G

 651 CTACTCCATGGGGGCTGGCTCTGATGCCGACATGGAGGCTGACACGGTGC
 107  Y  S  M  G  A  G  S  D  A  D  M  E  A  D  T  V  L

 701 TGTCCCCTGAGCACCCCGTGCGTCTGTGGGGCCGGAGCACACGGTCAGGG
 124   S  P  E  H  P  V  R  L  W  G  R  S  T  R  S  G

 751 CGCAGCTCCTGCCTGTCCAGCCGGGCCAATTCCAATCTCACACTCACCGA
 140 R  S  S  C  L  S  S  R  A  N  S  N  L  T  L  T  D

 801 CACCGAGCATGAAAACACTGAGACTGATCATCCGGGCGGCCTGCAGAACC
 157  T  E  H  E  N  T  E  T  D  H  P  G  G  L  Q  N  H

 851 ACGCGCGGCTCCGGACGCCGCCGCCGCCGCTCTCGCACGCCCACACCCCC
 174   A  R  L  R  T  P  P  P  P  L  S  H  A  H  T  P

 901 AACCAGCACCACGCGGCCTCCATTAACTCCCTGAACCGGGGCAACTTCAC
 190 N  Q  H  H  A  A  S  I  N  S  L  N  R  G  N  F  T

 951 GCCGAGGAGCAACCCCAGCCCGGCCCCCACGGACCACTCGCTCTCCGGAG
 207  P  R  S  N  P  S  P  A  P  T  D  H  S  L  S  G  E

1001 AGCCCCCTGCCGGCGGCGCCCAGGAGCCTGCCCACGCCCAGGAGAACTGG
 224   P  P  A  G  G  A  Q  E  P  A  H  A  Q  E  N  W

1051 CTGCTCAACAGCAACATCCCCCTGGAGACCAGAAACCTAGGCAAGCAGCC
 240 L  L  N  S  N  I  P  L  E  T  R  N  L  G  K  Q  P
```

FIG._7A

```
1101 ATTCCTAGGGACATTGCAGGACAACCTCATTGAGATGGACATTCTCGGCG
 257   F   L   G   T   L   Q   D   N   L   I   E   M   D   I   L   G   A

1151 CCTCCCGCCATGATGGGGCTTACAGTGACGGGCACTTCCTCTTCAAGCCT
 274     S   R   H   D   G   A   Y   S   D   G   H   F   L   F   K   P

1201 GGAGGCACCTCCCCGCTCTTCTGCACCACATCACCAGGGTACCCACTGAC
 290 G   G   T   S   P   L   F   C   T   T   S   P   G   Y   P   L   T

1251 GTCCAGCACAGTGTACTCTCCTCCGCCCCGACCCCTGCCCCGCAGCACCT
 307   S   S   T   V   Y   S   P   P   P   R   P   L   P   R   S   T   F

1301 TCGCCCGGCCGGCCTTTAACCTCAAGAAGCCCTCCAAGTACTGTAACTGG
 324     A   R   P   A   F   N   L   K   K   P   S   K   Y   C   N   W

1351 AAGTGCGCAGCCCTGAGCGCCATCGTCATCTCAGCCACTCTGGTCATCCT
 340 K   C   A   A   L   S   A   I   V   I   S   A   T   L   V   I   L

1401 GCTGGCATACTTTGTGGCCATGCACCTGTTTGGCCTAAACTGGCACCTGC
 357   L   A   Y   F   V   A   M   H   L   F   G   L   N   W   H   L   Q

1451 AGCCGATGGAGGGGCAGATGTATGAGATCACGGAGGACACAGCCAGCAGT
 374     P   M   E   G   Q   M   Y   E   I   T   E   D   T   A   S   S

1501 TGGCCTGTGCCAACCGACGTCTCCCTATACCCCTCAGGGGGCACTGGCTT
 390 W   P   V   P   T   D   V   S   L   Y   P   S   G   G   T   G   L

1551 AGAGACCCCTGACAGGAAAGGCAAAGGAACCACAGAAGGAAAGCCCAGTA
 407   E   T   P   D   R   K   G   K   G   T   T   E   G   K   P   S   S

1601 GTTTCTTTCCAGAGGACAGTTTCATAGATTCTGGAGAAATTGATGTGGGA
 424     F   F   P   E   D   S   F   I   D   S   G   E   I   D   V   G

1651 AGGCGAGCTTCCCAGAAGATTCCTCCTGGCACTTTCTGGAGATCTCAAGT
 440 R   R   A   S   Q   K   I   P   P   G   T   F   W   R   S   Q   V

1701 GTTCATAGACCATCCTGTGCATCTGAAATTCAATGTGTCTCTGGGAAAGG
 457   F   I   D   H   P   V   H   L   K   F   N   V   S   L   G   K   A

1751 CAGCCCTGGTTGGCATTTATGGCAGAAAAGGCCTCCCTCCTTCACATACA
 474     A   L   V   G   I   Y   G   R   K   G   L   P   P   S   H   T

1801 CAGTTTGACTTTGTGGAGCTGCTGGATGGCAGGAGGCTCCTAACCCAGGA
 490 Q   F   D   F   V   E   L   L   D   G   R   R   L   L   T   Q   E

1851 GGCGCGGAGCCTAGAGGGGACCCCGCGCCAGTCTCGGGGAACTGTGCCCC
 507   A   R   S   L   E   G   T   P   R   Q   S   R   G   T   V   P   P

1901 CCTCCAGCCATGAGACAGGCTTCATCCAGTATTTGGATTCAGGAATCTGG
 524     S   S   H   E   T   G   F   I   Q   Y   L   D   S   G   I   W

1951 CACTTGGCTTTTTACAATGACGGAAAGGAGTCAGAAGTGGTTTCCTTTCT
 540 H   L   A   F   Y   N   D   G   K   E   S   E   V   V   S   F   L
```

FIG._7B

```
2001 CACCACTGCCATTGCCTTGCCTCCCCGATTGAAAGAGATGAAAAGCCAGG
 557  T  T  A  I  A  L  P  P  R  L  K  E  M  K  S  Q  E

2051 AATCGGCTGCAGGTTCCAAACTAGTCCTTCGGTGTGAAACCAGTTCTGAA
 574   S  A  A  G  S  K  L  V  L  R  C  E  T  S  S  E

2101 TACTCCTCTCTCAGATTCAAGTGGTTCAAGAATGGGAATGAATTGAATCG
 590 Y  S  S  L  R  F  K  W  F  K  N  G  N  E  L  N  R

2151 AAAAAACAAACCACAAAATATCAAGATACAAAAAAAGCCAGGGAAGTCAG
 607  K  N  K  P  Q  N  I  K  I  Q  K  K  P  G  K  S  E

2201 AACTTCGCATTAACAAAGCATCACTGGCTGATTCTGGAGAGTATATGTGC
 624   L  R  I  N  K  A  S  L  A  D  S  G  E  Y  M  C

2251 AAAGTGATCAGCAAATTAGGAAATGACAGTGCCTCTGCCAATATCACCAT
 640 K  V  I  S  K  L  G  N  D  S  A  S  A  N  I  T  I

2301 CGTGGAATCAAACGAGATCATCACTGGTATGCCAGCCTCAACTGAAGGAG
 657  V  E  S  N  E  I  I  T  G  M  P  A  S  T  E  G  A

2351 CATATGTGTCTTCAGAGTCTCCCATTAGAATATCAGTATCCACAGAAGGA
 674   Y  V  S  S  E  S  P  I  R  I  S  V  S  T  E  G

2401 GCAAATACTTCTTCATCTACATCTACATCCACCACTGGGACAAGCCATCT
 690 A  N  T  S  S  S  T  S  T  S  T  T  G  T  S  H  L

2451 TGTAAAATGTGCGGAGAAGGAGAAAACTTTCTGTGTGAATGGAGGGGAGT
 707  V  K  C  A  E  K  E  K  T  F  C  V  N  G  G  E  C

2501 GCTTCATGGTGAAAGACCTTTCAAACCCCTCGAGATACTTGTGCAAGTGC
 724   F  M  V  K  D  L  S  N  P  S  R  Y  L  C  K  C

2551 CCAAATGAGTTTACTGGTGATCGCTGCCAAAACTACGTAATGGCCAGCTT
 740 P  N  E  F  T  G  D  R  C  Q  N  Y  V  M  A  S  F

2601 CTACAGTACGTCCACTCCCTTTCTGTCTCTGCCTGAATAGGAGCATGCTC
 757  Y  S  T  S  T  P  F  L  S  L  P  E

2651 AGTTGGTGCTGCTTTCTTGTTGCTGCATCTCCCCTCAGATTCCACCTAGA

2701 GCTAGATGTGTCTTACCAGATCTAATATTGACTGCCTCTGCCTGTCGCAT
2751 GAGAACATTAACAAAAGCAATTGTATTACTTCCTCTGTTCGCGACTAGTT
2801 GGCTCTGAGATACTAATAGGTGTGTGAGGCTCCGGATGTTTCTGGAATTG
2851 ATATTGAATGATGTGATACAAATTGATAGTCAATATCAAGCAGTGAAATA
2901 TGATAATAAAGGCATTTCAAAGTCTCACTTTTATTGATAAAATAAAAATC
2951 ATTCTACTGAACAGTCCATCTTCTTTATACAATGACCACATCCTGAAAAG
3001 GGTGTTGCTAAGCTGTAACCGATATGCACTTGAAATGATGGTAAGTTAAT
3051 TTTGATTCAGAATGTGTTATTTGTCACAAATAAACATAATAAAAGGAAAA
3101 AAAAAAAAAAA
```

FIG._7C

```
   1 GAATTCGGACAGCCTCTCCTGCCGCCGCTGCTGCCGCCGGCTGGTCCTCCTTCTGCTTT

  76 TACTTCTCCTGCATGACAGTTGTTTCTTCATCTGAGCAGACACCAGCTTCAGATGCTCGAGGTGAGAAACATGC

 151 CTTTCAGTTTGGGCTACTGGTTTACTTAATTAATCAGCCGGCAGCTCCGTCGATCTATTTTCGTCCCTGTCCTCT

 226 TGACGAGCCCGGGATGGTTTGGAGTAGCATTTAAAAGAACTAGAAAAGTGGCCCAGAAACAGCAGCTTAAAGAAT

 301 TATTACGATATACTTTGATTTTGTAGTTGCTAGGAGCTTTTTCTTCCCCCCTTGCATCTTTCTGAACTCTTCTTGA

 376 TTTTAATAATGGCCTTGGACTTGGACGATTTATCGATTTCCCCCTGTAAGATGCTGTATCATTTGGTTGGGGGGG

 451 CCTCTGCGTGGTAATGGACCGTGAGAGCGGCCAGGCCTTCTTCTGGAGGTGAGCCGATGGAGATTTATTCCCCAG
                                                    H  E  I  Y  S  P  D
   1

 526 ACATGTCTGAGGTCGCCGCCGAGAGGTCCTCCAGCCCCTCCACTCAGCTGAGTGCAGACCCATCTCTTGATGGGC
     H  S  E  V  A  A  E  E  R  S  S  S  P  S  T  Q  L  S  A  D  P  S  L  D  G  L
   8

 601 TTCCGGCAGCAGAAGACATGCCAGAGCCCCAGACTGAAGATGGGAGAACCCCTGGACTCGTGGGCCTGGCCGTGC
     P  A  A  E  D  H  P  E  P  Q  T  E  D  G  R  T  P  G  L  V  G  L  A  V  P
  33

 676 CCTGCTGTGCGTGCCTAGAAGCTGAGCGCCTGAGAGGTTGCCTCAACTCAGAGAAAATCTGCATTGTCCCCATCC
     C  C  A  C  L  E  A  E  R  L  R  G  C  L  N  S  E  K  I  C  I  V  P  I  L
  58

 751 TGGCTTGCCTGGTCAGCCTCTGCCTCTGCATCGCCGGCCTCAAGTGGGTATTTGTGGACAAGATCTTTGAATATG
     A  C  L  V  S  L  C  L  C  I  A  G  L  K  W  V  F  V  D  K  I  F  E  Y  D
  83

 826 ACTCTCCTACTCACCTTGACCCTGGGGGGTTAGGCCAGGACCCTATTATTTCTCTGGACGCAACTGCTGCCTCAG
 108 S  P  T  H  L  D  P  G  G  L  G  Q  D  P  I  I  S  L  D  A  T  A  A  S  A

 901 CTGTGTGGGTGTCGTCTGAGGCATACACTTCACCTGTCTCTAGGGCTCAATCTGAAGTGAGGTTCAAGTTACAG
 133 V  W  V  S  S  E  A  Y  T  S  P  V  S  R  A  Q  S  E  S  E  V  Q  V  T  V
```

FIG._8A

```
 976 TGCAAGGTGACAAGGCTGTTGTCTCCTTTGAACCATCAGCGGCACCGACACCGAAGAATCGTATTTTTGCCTTTT
 158   Q  G  D  K  A  V  V  S  F  E  P  S  A  A  P  T  P  K  N  R  I  F  A  F  S

1051 CTTTCTTGCCGTCCACTGCGCCATCCTTCCCTTCACCCACCCCGGAACCCTGAGGTGAGAACGCCCAAGTCAGCAA
 183   F  L  P  S  T  A  P  S  F  P  S  P  T  R  N  P  E  V  R  T  P  K  S  A  T

1126 CTCAGCCACAAACAACAGAAACTAATCTCCAAACTGCTCCTAAACTTTCTACATCTACATCCACCACTGGGACAA
 208   Q  P  Q  T  T  E  T  N  L  Q  T  A  P  K  L  S  T  S  T  S  T  T  G  T  S

1201 GCCATCTTGTAAAATGTGCGGAGAAGGAGAAAACTTTCTGTGTGAATGGAGGGGAGTGCTTCATGGTGAAAGACC
 233   H  L  V  K  C  A  E  K  E  K  T  F  C  V  N  G  G  E  C  F  M  V  K  D  L

1276 TTTCAAACCCCTCGAGATACTTGTGCAAGTGCCCAAATGAGTTTACTGGTGATCGCTGCCAAAACTACGTAATGG
 258   S  N  P  S  R  Y  L  C  K  C  P  N  E  F  T  G  D  R  C  Q  N  Y  V  M  A

1351 CCAGCTTCTACAGTACGTCCACTCCCTTTCTGTCTCTGCCTGAATAGGAGCATGCTCAGTTGGTGCTGCTTTCTT
 283   S  F  Y  S  T  S  T  P  F  L  S  L  P  E  O

1426 GTTGCTGCATCTCCCCTCAGATTCCACCTAGAGCTAGATGTGTCTTACCAGATCTAATATTGACTGCCTCTGCCT

1501 GTCGCATGAGAACATTAACAAAAGCAATTGTATTACTTCCTCTGTTCGCGACTAGTTGGCTCTGAGATACTAATA

1576 GGTGTGTGAGGCTCCGGATGTTTCTGGAATTGATATTGAATGATGTGATACAAATTGATAGTCAATATCAAGCAG

1651 TGAAATATGATAATAAAGGCATTTCAAAGTCTCACTTTTATTGATAAAATAAAAATCATTCTACTGAACAGTCCA

1726 TCTTCTTTATACAATGACCACATCCTGAAAAGGGTGTTGCTAAGCTGTAACCGATATGCACTTGAAATGATGGTA

1801 AGTTAATTTTGATTCAGAATGTGTTATTTGTCACAAATAAACATAATAAAAGGAAAAAAAAAACCCGAATTC
```

EGF-like

FIG._8B

Sequence Listing

<110> Genentech, Inc.

<120> Use of Heregulin as a Growth Factor

<130> P1145PCT

<141> 1999-02-03

<150> US 09/020,598
<151> 1998-02-04

<160> 14

<210> 1
<211> 669
<212> PRT
<213> Homo sapiens

<400> 1

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Ala Arg Ala Pro Gln Arg Gly Arg Ser Leu Ser Pro Ser Arg Asp
 1               5                  10                  15

Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu Gly Pro Asn Ser Pro
            20                  25                  30

Ala Pro Arg Ala Val Arg Val Glu Arg Ser Val Ser Gly Glu Met
        35                  40                  45

Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys Lys
    50                  55                  60

Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln
65                  70                  75

Ser Pro Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
            80                  85                  90

Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser
        95                  100                 105

Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu
    110                 115                 120

Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys
125                 130                 135

Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala Asp
            140                 145                 150

Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp
        155                 160                 165

Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile Ile
```

170 175 180

Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu
185 190 195

Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr Ser
200 205 210

Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys
215 220 225

Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
230 235 240

Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys
245 250 255

Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn Val Pro
260 265 270

Met Lys Val Gln Asn Gln Glu Lys Ala Glu Glu Leu Tyr Gln Lys
275 280 285

Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val Val
290 295 300

Gly Ile Met Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg
305 310 315

Lys Lys Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg
320 325 330

Asn Asn Met Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro
335 340 345

Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn
350 355 360

Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser
365 370 375

Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr Thr
380 385 390

Val Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu
395 400 405

Ser Ile Leu Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val
410 415 420

Glu Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg
425 430 435

Leu Asn Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg
440 445 450

```
His Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser
                455                 460                 465

Glu Arg Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro
                470                 475                 480

Val Asp Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro Ser Glu
                485                 490                 495

Met Ser Pro Pro Val Ser Ser Met Thr Val Ser Met Pro Ser Met
                500                 505                 510

Ala Val Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu Val
                515                 520                 525

Thr Pro Pro Arg Leu Arg Glu Lys Lys Phe Asp His His Pro Gln
                530                 535                 540

Gln Phe Ser Ser Phe His His Asn Pro Ala His Asp Ser Asn Ser
                545                 550                 555

Leu Pro Ala Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu
                560                 565                 570

Thr Thr Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys Leu
                575                 580                 585

Ala Asn Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn Gly His Ile
                590                 595                 600

Ala Asn Arg Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser
                605                 610                 615

Asn Ser Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp Thr
                620                 625                 630

Pro Phe Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu Ala
                635                 640                 645

Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala Gly
                650                 655                 660

Arg Phe Ser Thr Gln Glu Glu Ile Gln
                665                 669
```

<210> 2
<211> 2226
<212> DNA
<213> Homo sapiens

<400> 2

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gg  gcg cga gcg cct cag cgc ggc cgc tcg ctc tcc ccc 38
    Ala Arg Ala Pro Gln Arg Gly Arg Ser Leu Ser Pro
      1               5                  10

tcg agg gac aaa ctt ttc cca aac ccg atc cga gcc ctt 77
```

```
     Ser Arg Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu
               15                  20                  25

     gga cca aac tcg cct gcg ccg aga gcc gtc cgc gta gag 116
     Gly Pro Asn Ser Pro Ala Pro Arg Ala Val Arg Val Glu
 5                   30                  35

     cgc tcc gtc tcc ggc gag atg tcc gag cgc aaa gaa ggc 155
     Arg Ser Val Ser Gly Glu Met Ser Glu Arg Lys Glu Gly
          40                  45                  50

     aga ggc aaa ggg aag ggc aag aag aag gag cga ggc tcc 194
10   Arg Gly Lys Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser
                55                  60

     ggc aag aag ccg gag tcc gcg gcg ggc agc cag agc cca 233
     Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro
      65                  70                  75

15   gcc ttg cct ccc cga ttg aaa gag atg aaa agc cag gaa 272
     Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
               80                  85                  90

     tcg gct gca ggt tcc aaa cta gtc ctt cgg tgt gaa acc 311
     Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr
20                  95                  100

     agt tct gaa tac tcc tct ctc aga ttc aag tgg ttc aag 350
     Ser Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys
          105                 110                 115

     aat ggg aat gaa ttg aat cga aaa aac aaa cca caa aat 389
25   Asn Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn
               120                 125

     atc aag ata caa aaa aag cca ggg aag tca gaa ctt cgc 428
     Ile Lys Ile Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg
     130                 135                 140

30   att aac aaa gca tca ctg gct gat tct gga gag tat atg 467
     Ile Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met
               145                 150                 155

     tgc aaa gtg atc agc aaa tta gga aat gac agt gcc tct 506
     Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser
35                  160                 165

     gcc aat atc acc atc gtg gaa tca aac gag atc atc act 545
     Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile Ile Thr
          170                 175                 180

     ggt atg cca gcc tca act gaa gga gca tat gtg tct tca 584
40   Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
               185                 190

     gag tct ccc att aga ata tca gta tcc aca gaa gga gca 623
```

```
Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala
195                 200                 205

aat act tct tca tct aca tct aca tcc acc act ggg aca 662
Asn Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr
      210                 215                 220

agc cat ctt gta aaa tgt gcg gag aag gag aaa act ttc 701
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe
                225                 230

tgt gtg aat gga ggg gag tgc ttc atg gtg aaa gac ctt 740
Cys Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu
    235                 240                 245

tca aac ccc tcg aga tac ttg tgc aag tgc caa cct gga 779
Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys Gln Pro Gly
            250                 255

ttc act gga gca aga tgt act gag aat gtg ccc atg aaa 818
Phe Thr Gly Ala Arg Cys Thr Glu Asn Val Pro Met Lys
260                 265                 270

gtc caa aac caa gaa aag gcg gag gag ctg tac cag aag 857
Val Gln Asn Gln Glu Lys Ala Glu Glu Leu Tyr Gln Lys
      275                 280                 285

aga gtg ctg acc ata acc ggc atc tgc atc gcc ctc ctt 896
Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu
                290                 295

gtg gtc ggc atc atg tgt gtg gtg gcc tac tgc aaa acc 935
Val Val Gly Ile Met Cys Val Val Ala Tyr Cys Lys Thr
    300                 305                 310

aag aaa cag cgg aaa aag ctg cat gac cgt ctt cgg cag 974
Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln
            315                 320

agc ctt cgg tct gaa cga aac aat atg atg aac att gcc 1013
Ser Leu Arg Ser Glu Arg Asn Asn Met Met Asn Ile Ala
325                 330                 335

aat ggg cct cac cat cct aac cca ccc ccc gag aat gtc 1052
Asn Gly Pro His His Pro Asn Pro Pro Pro Glu Asn Val
      340                 345                 350

cag ctg gtg aat caa tac gta tct aaa aac gtc atc tcc 1091
Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile Ser
                355                 360

agt gag cat att gtt gag aga gaa gca gag aca tcc ttt 1130
Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser Phe
    365                 370                 375

tcc acc agt cac tat act tcc aca gcc cat cac tcc act 1169
```

```
Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr
            380                 385

act gtc acc cag act cct agc cac agc tgg agc aac gga 1208
Thr Val Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly
390                 395                 400

cac act gaa agc atc ctt tcc gaa agc cac tct gta atc 1247
His Thr Glu Ser Ile Leu Ser Glu Ser His Ser Val Ile
        405                 410                 415

gtg atg tca tcc gta gaa aac agt agg cac agc agc cca 1286
Val Met Ser Ser Val Glu Asn Ser Arg His Ser Ser Pro
                420                 425

act ggg ggc cca aga gga cgt ctt aat ggc aca gga ggc 1325
Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly Thr Gly Gly
    430                 435                 440

cct cgt gaa tgt aac agc ttc ctc agg cat gcc aga gaa 1364
Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala Arg Glu
            445                 450

acc cct gat tcc tac cga gac tct cct cat agt gaa agg 1403
Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg
455                 460                 465

tat gtg tca gcc atg acc acc ccg gct cgt atg tca cct 1442
Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro
        470                 475                 480

gta gat ttc cac acg cca agc tcc ccc aaa tcg ccc cct 1481
Val Asp Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro
                485                 490

tcg gaa atg tct cca ccc gtg tcc agc atg acg gtg tcc 1520
Ser Glu Met Ser Pro Pro Val Ser Ser Met Thr Val Ser
    495                 500                 505

atg cct tcc atg gcg gtc agc ccc ttc atg gaa gaa gag 1559
Met Pro Ser Met Ala Val Ser Pro Phe Met Glu Glu Glu
            510                 515

aga cct cta ctt ctc gtg aca cca cca agg ctg cgg gag 1598
Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu Arg Glu
520                 525                 530

aag aag ttt gac cat cac cct cag cag ttc agc tcc ttc 1637
Lys Lys Phe Asp His His Pro Gln Gln Phe Ser Ser Phe
        535                 540                 545

cac cac aac ccc gcg cat gac agt aac agc ctc cct gct 1676
His His Asn Pro Ala His Asp Ser Asn Ser Leu Pro Ala
                550                 555

agc ccc ttg agg ata gtg gag gat gag gag tat gaa acg 1715
```

```
Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu Thr
    560                 565                 570

acc caa gag tac gag cca gcc caa gag cct gtt aag aaa 1754
Thr Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys
             575                 580

ctc gcc aat agc cgg cgg gcc aaa aga acc aag ccc aat 1793
Leu Ala Asn Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn
585                 590                 595

ggc cac att gct aac aga ttg gaa gtg gac agc aac aca 1832
Gly His Ile Ala Asn Arg Leu Glu Val Asp Ser Asn Thr
        600                 605                 610

agc tcc cag agc agt aac tca gag agt gaa aca gaa gat 1871
Ser Ser Gln Ser Ser Asn Ser Glu Ser Glu Thr Glu Asp
                615                 620

gaa aga gta ggt gaa gat acg cct ttc ctg ggc ata cag 1910
Glu Arg Val Gly Glu Asp Thr Pro Phe Leu Gly Ile Gln
    625                 630                 635

aac ccc ctg gca gcc agt ctt gag gca aca cct gcc ttc 1949
Asn Pro Leu Ala Ala Ser Leu Glu Ala Thr Pro Ala Phe
             640                 645

cgc ctg gct gac agc agg act aac cca gca ggc cgc ttc 1988
Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala Gly Arg Phe
650                 655                 660

tcg aca cag gaa gaa atc cag g ccaggctgtc tagtgtaatt 2030
Ser Thr Gln Glu Glu Ile Gln
        665             669

gctaaccaag accctattgc tgtataaaac ctaaataaac acatagattc 2080

acctgtaaaa ctttatttta tataataaag tattccacct taaattaaac 2130

aatttatttt attttagcag ttctgcaaat agaaaacagg aaaaaaactt 2180

ttataaatta aatatatgta tgtaaaaatg aaaaaaaaaa aaaaaa 2226
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<210> 3
<211> 675
<212> PRT
<213> Homo sapiens

<400> 3

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Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu Gly Pro Asn Ser
  1               5                  10                  15

Pro Ala Pro Arg Ala Val Arg Val Glu Arg Ser Val Ser Gly Glu
                 20                  25                  30

Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys
```

```
                    35                  40                  45

Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
                    50                  55                  60

Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln
                    65                  70                  75

Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
                    80                  85                  90

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
                    95                 100                 105

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
                   110                 115                 120

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
                   125                 130                 135

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
                   140                 145                 150

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
                   155                 160                 165

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
                   170                 175                 180

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
                   185                 190                 195

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
                   200                 205                 210

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
                   215                 220                 225

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
                   230                 235                 240

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
                   245                 250                 255

Met Ala Ser Phe Tyr Lys His Leu Gly Ile Glu Phe Met Glu Ala
                   260                 265                 270

Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys
                   275                 280                 285

Ile Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr Cys
                   290                 295                 300

Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln
                   305                 310                 315
```

```
Ser Leu Arg Ser Glu Arg Asn Asn Met Met Asn Ile Ala Asn Gly
                320                 325                 330

Pro His His Pro Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn
                335                 340                 345

Gln Tyr Val Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu
                350                 355                 360

Arg Glu Ala Glu Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr
                365                 370                 375

Ala His His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser Trp
                380                 385                 390

Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser His Ser Val
                395                 400                 405

Ile Val Met Ser Ser Val Glu Asn Ser Arg His Ser Ser Pro Thr
                410                 415                 420

Gly Gly Pro Arg Gly Arg Leu Asn Gly Thr Gly Gly Pro Arg Glu
                425                 430                 435

Cys Asn Ser Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr
                440                 445                 450

Arg Asp Ser Pro His Ser Glu Arg Tyr Val Ser Ala Met Thr Thr
                455                 460                 465

Pro Ala Arg Met Ser Pro Val Asp Phe His Thr Pro Ser Ser Pro
                470                 475                 480

Lys Ser Pro Pro Ser Glu Met Ser Pro Pro Val Ser Ser Met Thr
                485                 490                 495

Val Ser Met Pro Ser Met Ala Val Ser Pro Phe Met Glu Glu Glu
                500                 505                 510

Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu Arg Glu Lys Lys
                515                 520                 525

Phe Asp His His Pro Gln Gln Phe Ser Ser Phe His His Asn Pro
                530                 535                 540

Ala His Asp Ser Asn Ser Leu Pro Ala Ser Pro Leu Arg Ile Val
                545                 550                 555

Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln
                560                 565                 570

Glu Pro Val Lys Lys Leu Ala Asn Ser Arg Arg Ala Lys Arg Thr
                575                 580                 585

Lys Pro Asn Gly His Ile Ala Asn Arg Leu Glu Val Asp Ser Asn
                590                 595                 600
```

```
Thr Ser Ser Gln Ser Ser Asn Ser Glu Ser Glu Thr Glu Asp Glu
                605                 610                 615

Arg Val Gly Glu Asp Thr Pro Phe Leu Gly Ile Gln Asn Pro Leu
                620                 625                 630

Ala Ala Ser Leu Glu Ala Thr Pro Ala Phe Arg Leu Ala Asp Ser
                635                 640                 645

Arg Thr Asn Pro Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile Gln
                650                 655                 660

Ala Arg Leu Ser Ser Val Ile Ala Asn Gln Asp Pro Ile Ala Val
                665                 670                 675
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    Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu Gly
      1               5                  10

cca aac tcg cct gcg ccg aga gcc gtc cgc gta gag cgc 77
Pro Asn Ser Pro Ala Pro Arg Ala Val Arg Val Glu Arg
          15                  20                  25

tcc gtc tcc ggc gag atg tcc gag cgc aaa gaa ggc aga 116
Ser Val Ser Gly Glu Met Ser Glu Arg Lys Glu Gly Arg
                30                  35

ggc aaa ggg aag ggc aag aag aag gag cga ggc tcc ggc 155
Gly Lys Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser Gly
     40                  45                  50

aag aag ccg gag tcc gcg gcg ggc agc cag agc cca gcc 194
Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro Ala
          55                  60

ttg cct ccc caa ttg aaa gag atg aaa agc cag gaa tcg 233
Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser
 65                  70                  75

gct gca ggt tcc aaa cta gtc ctt cgg tgt gaa acc agt 272
Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
          80                  85                  90

tct gaa tac tcc tct ctc aga ttc aag tgg ttc aag aat 311
Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn
                95                  100

ggg aat gaa ttg aat cga aaa aac aaa cca caa aat atc 350
Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile
    105                 110                 115
```

```
aag ata caa aaa aag cca ggg aag tca gaa ctt cgc att 389
Lys Ile Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile
            120                 125

aac aaa gca tca ctg gct gat tct gga gag tat atg tgc 428
Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys
130                 135                 140

aaa gtg atc agc aaa tta gga aat gac agt gcc tct gcc 467
Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala
        145                 150                 155

aat atc acc atc gtg gaa tca aac gag atc atc act ggt 506
Asn Ile Thr Ile Val Glu Ser Asn Glu Ile Ile Thr Gly
                160                 165

atg cca gcc tca act gaa gga gca tat gtg tct tca gag 545
Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu
    170                 175                 180

tct ccc att aga ata tca gta tcc aca gaa gga gca aat 584
Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn
            185                 190

act tct tca tct aca tct aca tcc acc act ggg aca agc 623
Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser
195                 200                 205

cat ctt gta aaa tgt gcg gag aag gag aaa act ttc tgt 662
His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys
        210                 215                 220

gtg aat gga ggg gag tgc ttc atg gtg aaa gac ctt tca 701
Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser
                225                 230

aac ccc tcg aga tac ttg tgc aag tgc cca aat gag ttt 740
Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe
    235                 240                 245

act ggt gat cgc tgc caa aac tac gta atg gcc agc ttc 779
Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe
            250                 255

tac aag cat ctt ggg att gaa ttt atg gag gcg gag gag 818
Tyr Lys His Leu Gly Ile Glu Phe Met Glu Ala Glu Glu
260                 265                 270

ctg tac cag aag aga gtg ctg acc ata acc ggc atc tgc 857
Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys
        275                 280                 285

atc gcc ctc ctt gtg gtc ggc atc atg tgt gtg gtg gcc 896
Ile Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala
                290                 295
```

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tac tgc aaa acc aag aaa cag cgg aaa aag ctg cat gac 935
Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp
    300                 305                 310

cgt ctt cgg cag agc ctt cgg tct gaa cga aac aat atg 974
Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn Met
            315                 320

atg aac att gcc aat ggg cct cac cat cct aac cca ccc 1013
Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro
325                 330                 335

ccc gag aat gtc cag ctg gtg aat caa tac gta tct aaa 1052
Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys
        340                 345                 350

aac gtc atc tcc agt gag cat att gtt gag aga gaa gca 1091
Asn Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala
                355                 360

gag aca tcc ttt tcc acc agt cac tat act tcc aca gcc 1130
Glu Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala
    365                 370                 375

cat cac tcc act act gtc acc cag act cct agc cac agc 1169
His His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser
            380                 385

tgg agc aac gga cac act gaa agc atc ctt tcc gaa agc 1208
Trp Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser
390                 395                 400

cac tct gta atc gtg atg tca tcc gta gaa aac agt agg 1247
His Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg
        405                 410                 415

cac agc agc cca act ggg ggc cca aga gga cgt ctt aat 1286
His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
                420                 425

ggc aca gga ggc cct cgt gaa tgt aac agc ttc ctc agg 1325
Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg
    430                 435                 440

cat gcc aga gaa acc cct gat tcc tac cga gac tct cct 1364
His Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro
            445                 450

cat agt gaa agg tat gtg tca gcc atg acc acc ccg gct 1403
His Ser Glu Arg Tyr Val Ser Ala Met Thr Thr Pro Ala
455                 460                 465

cgt atg tca cct gta gat ttc cac acg cca agc tcc ccc 1442
Arg Met Ser Pro Val Asp Phe His Thr Pro Ser Ser Pro
        470                 475                 480
```

```
aaa tcg ccc cct tcg gaa atg tct cca ccc gtg tcc agc 1481
Lys Ser Pro Pro Ser Glu Met Ser Pro Pro Val Ser Ser
                485                 490

atg acg gtg tcc atg cct tcc atg gcg gtc agc ccc ttc 1520
Met Thr Val Ser Met Pro Ser Met Ala Val Ser Pro Phe
    495                 500                 505

atg gaa gaa gag aga cct cta ctt ctc gtg aca cca cca 1559
Met Glu Glu Glu Arg Pro Leu Leu Leu Val Thr Pro Pro
            510                 515

agg ctg cgg gag aag aag ttt gac cat cac cct cag cag 1598
Arg Leu Arg Glu Lys Lys Phe Asp His His Pro Gln Gln
520                 525                 530

ttc agc tcc ttc cac cac aac ccc gcg cat gac agt aac 1637
Phe Ser Ser Phe His His Asn Pro Ala His Asp Ser Asn
        535                 540                 545

agc ctc cct gct agc ccc ttg agg ata gtg gag gat gag 1676
Ser Leu Pro Ala Ser Pro Leu Arg Ile Val Glu Asp Glu
                550                 555

gag tat gaa acg acc caa gag tac gag cca gcc caa gag 1715
Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln Glu
    560                 565                 570

cct gtt aag aaa ctc gcc aat agc cgg cgg gcc aaa aga 1754
Pro Val Lys Lys Leu Ala Asn Ser Arg Arg Ala Lys Arg
            575                 580

acc aag ccc aat ggc cac att gct aac aga ttg gaa gtg 1793
Thr Lys Pro Asn Gly His Ile Ala Asn Arg Leu Glu Val
585                 590                 595

gac agc aac aca agc tcc cag agc agt aac tca gag agt 1832
Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn Ser Glu Ser
        600                 605                 610

gaa aca gaa gat gaa aga gta ggt gaa gat acg cct ttc 1871
Glu Thr Glu Asp Glu Arg Val Gly Glu Asp Thr Pro Phe
                615                 620

ctg ggc ata cag aac ccc ctg gca gcc agt ctt gag gca 1910
Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu Ala
    625                 630                 635

aca cct gcc ttc cgc ctg gct gac agc agg act aac cca 1949
Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro
            640                 645

gca ggc cgc ttc tcg aca cag gaa gaa atc cag gcc agg 1988
Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile Gln Ala Arg
650                 655                 660
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ctg tct agt gta att gct aac caa gac cct att gct gta ta 2029
Leu Ser Ser Val Ile Ala Asn Gln Asp Pro Ile Ala Val
665 670 675

a aacctaaata aacacataga ttcacctgta aaactttatt 2070

ttatataata aagtattcca ccttaaatta aacaatttat tttattttag 2120

cagttctgca aatagaaaac aggaaaaaaa cttttataaa ttaaatatat 2170

gtatgtaaaa atgaaaaaaa aaaaaaaaa 2199

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Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
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Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln
35 40 45

Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
50 55 60

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
65 70 75

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
80 85 90

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
95 100 105

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
110 115 120

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
125 130 135

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
140 145 150

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
155 160 165

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
170 175 180

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
185 190 195

```
Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
                200                 205                 210

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
                215                 220                 225

Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val
                230                 235                 240

Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val Val Gly Ile
                245                 250                 255

Met Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys
                260                 265                 270

Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn
                275                 280                 285

Met Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
                290                 295                 300

Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile
                305                 310                 315

Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser Phe Ser
                320                 325                 330

Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr Thr Val Thr
                335                 340                 345

Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile
                350                 355                 360

Leu Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu Asn
                365                 370                 375

Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
                380                 385                 390

Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala
                395                 400                 405

Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg
                410                 415                 420

Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro Val Asp
                425                 430                 435

Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro Ser Glu Met Ser
                440                 445                 450

Pro Pro Val Ser Ser Met Thr Val Ser Lys Pro Ser Met Ala Val
                455                 460                 465

Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu Val Thr Pro
                470                 475                 480
```

Pro Arg Leu Arg Glu Lys Lys Phe Asp His His Pro Gln Gln Phe
485 490 495

Ser Ser Phe His His Asn Pro Ala His Asp Ser Asn Ser Leu Pro
500 505 510

Ala Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu Thr Thr
515 520 525

Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys Leu Ala Asn
530 535 540

Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn Gly His Ile Ala Asn
545 550 555

Arg Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn Ser
560 565 570

Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp Thr Pro Phe
575 580 585

Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu Ala Thr Pro
590 595 600

Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala Gly Arg Phe
605 610 615

Ser Thr Gln Glu Glu Ile Gln Ala Arg Leu Ser Ser Val Ile Ala
620 625 630

Asn Gln Asp Pro Ile Ala Val
635 637

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ccggacgatg ggagcgtgag caggacggtg ataacctctc cccgatcggg 150

ttgcgagggc gccgggcaga ggccaggacg cgagccgcca gcggcgggac 200

ccatcgacga cttcccgggg cgacaggagc agccccgaga gccagggcga 250

gcgcccgttc caggtggccg gaccgcccgc cgcgtccgcg ccgcgctccc 300

tgcaggcaac gggagacgcc cccgcgcagc gcgagcgcct cagcgcggcc 350

gctcgctctc cccatcgagg gacaaacttt tcccaaaccc gatccgagcc 400

cttggaccaa actcgcctgc gccgagagcc gtccgcgtag agcgctccgt 450

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ctccggcgag  atg tcc gag cgc aaa gaa ggc aga ggc aaa 490
            Met Ser Glu Arg Lys Glu Gly Arg Gly Lys
              1               5                  10

ggg aag ggc aag aag aag gag cga ggc tcc ggc aag aag 529
Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser Gly Lys Lys
                  15                  20

ccg gag tcc gcg gcg ggc agc cag agc cca gcc ttg cct 568
Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro Ala Leu Pro
     25                  30                  35

ccc caa ttg aaa gag atg aaa agc cag gaa tcg gct gca 607
Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser Ala Ala
              40                  45

ggt tcc aaa cta gtc ctt cgg tgt gaa acc agt tct gaa 646
Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
 50                  55                  60

tac tcc tct ctc aga ttc aag tgg ttc aag aat ggg aat 685
Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
          65                  70                  75

gaa ttg aat cga aaa aac aaa cca caa aat atc aag ata 724
Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile
                  80                  85

caa aaa aag cca ggg aag tca gaa ctt cgc att aac aaa 763
Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys
     90                  95                 100

gca tca ctg gct gat tct gga gag tat atg tgc aaa gtg 802
Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val
             105                 110

atc agc aaa tta gga aat gac agt gcc tct gcc aat atc 841
Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile
115                 120                 125

acc atc gtg gaa tca aac gag atc atc act ggt atg cca 880
Thr Ile Val Glu Ser Asn Glu Ile Ile Thr Gly Met Pro
         130                 135                 140

gcc tca act gaa gga gca tat gtg tct tca gag tct ccc 919
Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu Ser Pro
                 145                 150

att aga ata tca gta tcc aca gaa gga gca aat act tct 958
Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr Ser
    155                 160                 165

tca tct aca tct aca tcc acc act ggg aca agc cat ctt 997
Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu
             170                 175
```

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gta aaa tgt gcg gag aag gag aaa act ttc tgt gtg aat 1036
Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
180                 185                 190

gga ggg gag tgc ttc atg gtg aaa gac ctt tca aac ccc 1075
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro
        195                 200                 205

tcg aga tac ttg tgc aag tgc cca aat gag ttt act ggt 1114
Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly
                210                 215

gat cgc tgc caa aac tac gta atg gcc agc ttc tac aag 1153
Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys
    220                 225                 230

gcg gag gag ctg tac cag aag aga gtg ctg acc ata acc 1192
Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr
            235                 240

ggc atc tgc atc gcc ctc ctt gtg gtc ggc atc atg tgt 1231
Gly Ile Cys Ile Ala Leu Leu Val Val Gly Ile Met Cys
245                 250                 255

gtg gtg gcc tac tgc aaa acc aag aaa cag cgg aaa aag 1270
Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys
        260                 265                 270

ctg cat gac cgt ctt cgg cag agc ctt cgg tct gaa cga 1309
Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg
                275                 280

aac aat atg atg aac att gcc aat ggg cct cac cat cct 1348
Asn Asn Met Met Asn Ile Ala Asn Gly Pro His His Pro
    285                 290                 295

aac cca ccc ccc gag aat gtc cag ctg gtg aat caa tac 1387
Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr
            300                 305

gta tct aaa aac gtc atc tcc agt gag cat att gtt gag 1426
Val Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu
310                 315                 320

aga gaa gca gag aca tcc ttt tcc acc agt cac tat act 1465
Arg Glu Ala Glu Thr Ser Phe Ser Thr Ser His Tyr Thr
        325                 330                 335

tcc aca gcc cat cac tcc act act gtc acc cag act cct 1504
Ser Thr Ala His His Ser Thr Thr Val Thr Gln Thr Pro
                340                 345

agc cac agc tgg agc aac gga cac act gaa agc atc ctt 1543
Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile Leu
    350                 355                 360
```

```
tcc gaa agc cac tct gta atc gtg atg tca tcc gta gaa 1582
Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu
            365                 370

aac agt agg cac agc agc cca act ggg ggc cca aga gga 1621
Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly
375                 380                 385

cgt ctt aat ggc aca gga ggc cct cgt gaa tgt aac agc 1660
Arg Leu Asn Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser
        390                 395                 400

ttc ctc agg cat gcc aga gaa acc cct gat tcc tac cga 1699
Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr Arg
                405                 410

gac tct cct cat agt gaa agg tat gtg tca gcc atg acc 1738
Asp Ser Pro His Ser Glu Arg Tyr Val Ser Ala Met Thr
    415                 420                 425

acc ccg gct cgt atg tca cct gta gat ttc cac acg cca 1777
Thr Pro Ala Arg Met Ser Pro Val Asp Phe His Thr Pro
            430                 435

agc tcc ccc aaa tcg ccc cct tcg gaa atg tct cca ccc 1816
Ser Ser Pro Lys Ser Pro Pro Ser Glu Met Ser Pro Pro
440                 445                 450

gtg tcc agc atg acg gtg tcc aag cct tcc atg gcg gtc 1855
Val Ser Ser Met Thr Val Ser Lys Pro Ser Met Ala Val
        455                 460                 465

agc ccc ttc atg gaa gaa gag aga cct cta ctt ctc gtg 1894
Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu Val
                470                 475

aca cca cca agg ctg cgg gag aag aag ttt gac cat cac 1933
Thr Pro Pro Arg Leu Arg Glu Lys Lys Phe Asp His His
    480                 485                 490

cct cag cag ttc agc tcc ttc cac cac aac ccc gcg cat 1972
Pro Gln Gln Phe Ser Ser Phe His His Asn Pro Ala His
            495                 500

gac agt aac agc ctc cct gct agc ccc ttg agg ata gtg 2011
Asp Ser Asn Ser Leu Pro Ala Ser Pro Leu Arg Ile Val
505                 510                 515

gag gat gag gag tat gaa acg acc caa gag tac gag cca 2050
Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro
        520                 525                 530

gcc caa gag cct gtt aag aaa ctc gcc aat agc cgg cgg 2089
Ala Gln Glu Pro Val Lys Lys Leu Ala Asn Ser Arg Arg
                535                 540
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gcc aaa aga acc aag ccc aat ggc cac att gct aac aga 2128
Ala Lys Arg Thr Lys Pro Asn Gly His Ile Ala Asn Arg
545 550 555

ttg gaa gtg gac agc aac aca agc tcc cag agc agt aac 2167
Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn
560 565

tca gag agt gaa aca gaa gat gaa aga gta ggt gaa gat 2206
Ser Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp
570 575 580

acg cct ttc ctg ggc ata cag aac ccc ctg gca gcc agt 2245
Thr Pro Phe Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser
585 590 595

ctt gag gca aca cct gcc ttc cgc ctg gct gac agc agg 2284
Leu Glu Ala Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg
600 605

act aac cca gca ggc cgc ttc tcg aca cag gaa gaa atc 2323
Thr Asn Pro Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile
610 615 620

cag gcc agg ctg tct agt gta att gct aac caa gac cct 2362
Gln Ala Arg Leu Ser Ser Val Ile Ala Asn Gln Asp Pro
625 630

att gct gta taaaaccta ataaaacaca tagattcacc tgtaaaactt 2410
Ile Ala Val
635 637

tattttatat aataaagtat tccaccttaa attaaacaat ttatttttatt 2460

ttagcagttc tgcaaataaa aaaaaaaaaa 2490

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<212> PRT
<213> Homo sapiens

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Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
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Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln
35 40 45

Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
50 55 60

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
65 70 75

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
80 85 90

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
95 100 105

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
110 115 120

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
125 130 135

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
140 145 150

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
155 160 165

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
170 175 180

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
185 190 195

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
200 205 210

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
215 220 225

Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro
230 235 240

Glu
241

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<211> 1715
<212> DNA
<213> Homo sapiens

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cgcggaggcc aggagctgag cggcggcggc tgccggacga tgggagcgtg 150

agcaggacgg tgataacctc tccccgatcg ggttgcgagg gcgccgggca 200

gaggccagga cgcgagccgc cagcggcggg acccatcgac gacttcccgg 250

ggcgacagga gcagccccga gagccagggc gagcgcccgt tccaggtggc 300

cggaccgccc gccgcgtccg cgccgcgctc cctgcaggca acgggagacg 350

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ccccgcgca gcgcgagcgc ctcagcgcgg ccgctcgctc tccccatcga 400

gggacaaact tttcccaaac ccgatccgag cccttggacc aaactcgcct 450

gcgccgagag ccgtccgcgt agagcgctcc gtctccggcg ag  atg 495
                                                Met
                                                  1

tcc gag cgc aaa gaa ggc aga ggc aaa ggg aag ggc aag 534
Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys
             5                  10

aag aag gag cga ggc tcc ggc aag aag ccg gag tcc gcg 573
Lys Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala
 15                  20                  25

gcg ggc agc cag agc cca gcc ttg cct ccc caa ttg aaa 612
Ala Gly Ser Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys
          30                  35                  40

gag atg aaa agc cag gaa tcg gct gca ggt tcc aaa cta 651
Glu Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu
                45                  50

gtc ctt cgg tgt gaa acc agt tct gaa tac tcc tct ctc 690
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
     55                  60                  65

aga ttc aag tgg ttc aag aat ggg aat gaa ttg aat cga 729
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg
             70                  75

aaa aac aaa cca caa aat atc aag ata caa aaa aag cca 768
Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro
 80                  85                  90

ggg aag tca gaa ctt cgc att aac aaa gca tca ctg gct 807
Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
          95                 100                 105

gat tct gga gag tat atg tgc aaa gtg atc agc aaa tta 846
Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
                110                 115

gga aat gac agt gcc tct gcc aat atc acc atc gtg gaa 885
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu
     120                 125                 130

tca aac gag atc atc act ggt atg cca gcc tca act gaa 924
Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
             135                 140

gga gca tat gtg tct tca gag tct ccc att aga ata tca 963
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser
145                 150                 155
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gta tcc aca gaa gga gca aat act tct tca tct aca tct 1002
Val Ser Thr Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser
        160                 165                 170

aca tcc acc act ggg aca agc cat ctt gta aaa tgt gcg 1041
Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala
                175                 180

gag aag gag aaa act ttc tgt gtg aat gga ggg gag tgc 1080
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
    185                 190                 195

ttc atg gtg aaa gac ctt tca aac ccc tcg aga tac ttg 1119
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
            200                 205

tgc aag tgc cca aat gag ttt act ggt gat cgc tgc caa 1158
Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln
210                 215                 220

aac tac gta atg gcc agc ttc tac agt acg tcc act ccc 1197
Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro
        225                 230                 235

ttt ctg tct ctg cct gaa tagga gcatgctcag ttggtgctgc 1240
Phe Leu Ser Leu Pro Glu
                240 241

tttcttgttg ctgcatctcc cctcagattc cacctagagc tagatgtgtc 1290

ttaccagatc taatattgac tgcctctgcc tgtcgcatga gaacattaac 1340

aaaagcaatt gtattacttc ctctgttcgc gactagttgg ctctgagata 1390

ctaataggtg tgtgaggctc cggatgtttc tggaattgat attgaatgat 1440

gtgatacaaa ttgatagtca atatcaagca gtgaaatatg ataataaagg 1490

catttcaaag tctcactttt attgataaaa taaaaatcat tctactgaac 1540

agtccatctt ctttatacaa tgaccacatc ctgaaaaggg tgttgctaag 1590

ctgtaaccga tatgcacttg aaatgatggt aagttaattt tgattcagaa 1640

tgtgttattt gtcacaaata aacataataa aaggagttca gatgtttttc 1690

ttcattaacc aaaaaaaaaa aaaaa 1715

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<211> 420
<212> PRT
<213> Homo sapiens

<400> 9
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   1               5                  10                  15
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Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
                 20                  25                  30

Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln
                 35                  40                  45

Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
                 50                  55                  60

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
                 65                  70                  75

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
                 80                  85                  90

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
                 95                 100                 105

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
                110                 115                 120

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
                125                 130                 135

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
                140                 145                 150

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
                155                 160                 165

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
                170                 175                 180

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
                185                 190                 195

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
                200                 205                 210

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
                215                 220                 225

Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val
                230                 235                 240

Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val Val Gly Ile
                245                 250                 255

Met Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys
                260                 265                 270

Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn
                275                 280                 285

Met Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
                290                 295                 300
```

Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile
305 310 315

Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser Phe Ser
320 325 330

Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr Thr Val Thr
335 340 345

Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile
350 355 360

Leu Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu Asn
365 370 375

Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
380 385 390

Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala
395 400 405

Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg
410 415 420

<210> 10
<211> 2431
<212> DNA
<213> Homo sapiens

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ttgaaaaaga gccggcgagg agttccccga aacttgttgg aactccgggc 100

tcgcgcggag gccaggagct gagcggcggc ggctgccgga cgatgggagc 150

gtgagcagga cggtgataac ctctccccga tcgggttgcg agggcgccgg 200

gcagaggcca ggacgcgagc cgccagcggc gggacccatc gacgacttcc 250

cggggcgaca ggagcagccc cgagagccag ggcgagcgcc cgttccaggt 300

ggccggaccg cccgccgcgt ccgcgccgcg ctccctgcag gcaacgggag 350

acgcccccgc gcagcgcgag cgcctcagcg cggccgctcg ctctccccat 400

cgagggacaa acttttccca aacccgatcc gagcccttgg accaaactcg 450

cctgcgccga gagccgtccg cgtagagcgc tccgtctccg gcgag atg 498
Met
1

tcc gag cgc aaa gaa ggc aga ggc aaa ggg aag ggc aag 537
Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys
5 10

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aag aag gag cga ggc tcc ggc aag aag ccg gag tcc gcg 576
Lys Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala
 15                  20                  25

gcg ggc agc cag agc cca gcc ttg cct ccc caa ttg aaa 615
Ala Gly Ser Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys
          30                  35                  40

gag atg aaa agc cag gaa tcg gct gca ggt tcc aaa cta 654
Glu Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu
                    45                  50

gtc ctt cgg tgt gaa acc agt tct gaa tac tcc tct ctc 693
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
     55                  60                  65

aga ttc aag tgg ttc aag aat ggg aat gaa ttg aat cga 732
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg
               70                  75

aaa aac aaa cca caa aat atc aag ata caa aaa aag cca 771
Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro
 80                  85                  90

ggg aag tca gaa ctt cgc att aac aaa gca tca ctg gct 810
Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
          95                  100                 105

gat tct gga gag tat atg tgc aaa gtg atc agc aaa tta 849
Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
                    110                 115

gga aat gac agt gcc tct gcc aat atc acc atc gtg gaa 888
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu
     120                 125                 130

tca aac gag atc atc act ggt atg cca gcc tca act gaa 927
Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
               135                 140

gga gca tat gtg tct tca gag tct ccc att aga ata tca 966
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser
145                 150                 155

gta tcc aca gaa gga gca aat act tct tca tct aca tct 1005
Val Ser Thr Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser
          160                 165                 170

aca tcc acc act ggg aca agc cat ctt gta aaa tgt gcg 1044
Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala
                    175                 180

gag aag gag aaa act ttc tgt gtg aat gga ggg gag tgc 1083
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
     185                 190                 195
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ttc atg gtg aaa gac ctt tca aac ccc tcg aga tac ttg 1122
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
            200                 205

tgc aag tgt cca aat gag ttt act ggt gat cgc tgc caa 1161
Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln
210                 215                 220

aac tac gta atg gcc agc ttc tac aag gcg gag gag ctg 1200
Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu
        225                 230                 235

tac cag aag aga gtg ctg acc ata acc ggc atc tgc atc 1239
Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile
                240                 245

gcc ctc ctt gtg gtc ggc atc atg tgt gtg gtg gcc tac 1278
Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr
    250                 255                 260

tgc aaa acc aag aaa cag cgg aaa aag ctg cat gac cgt 1317
Cys Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg
            265                 270

ctt cgg cag agc ctt cgg tct gaa cga aac aat atg atg 1356
Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn Met Met
275                 280                 285

aac att gcc aat ggg cct cac cat cct aac cca ccc ccc 1395
Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
        290                 295                 300

gag aat gtc cag ctg gtg aat caa tac gta tct aaa aac 1434
Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn
                305                 310

gtc atc tcc agt gag cat att gtt gag aga gaa gca gag 1473
Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu
    315                 320                 325

aca tcc ttt tcc acc agt cac tat act tcc aca gcc cat 1512
Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His
            330                 335

cac tcc act act gtc acc cag act cct agc cac agc tgg 1551
His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser Trp
340                 345                 350

agc aac gga cac act gaa agc atc ctt tcc gaa agc cac 1590
Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser His
        355                 360                 365

tct gta atc gtg atg tca tcc gta gaa aac agt agg cac 1629
Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg His
                370                 375
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agc agc cca act ggg ggc cca aga gga cgt ctt aat ggc 1668
Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly
380 385 390

aca gga ggc cct cgt gaa tgt aac agc ttc ctc agg cat 1707
Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His
395 400

gcc aga gaa acc cct gat tcc tac cga gac tct cct cat 1746
Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His
405 410 415

agt gaa agg taaaa ccgaaggcaa agctactgca gaggagaaac 1790
Ser Glu Arg
420

tcagtcagag aatccctgtg agcacctgcg gtctcacctc aggaaatcta 1840

ctctaatcag aataaggggc ggcagttacc tgttctagga gtgctcctag 1890

ttgatgaagt catctctttg tttgacggaa cttatttctt ctgagcttct 1940

ctcgtcgtcc cagtgactga caggcaacag actcttaaag agctgggatg 1990

ctttgatgcg gaaggtgcag cacatggagt ttccagctct ggccatgggc 2040

tcagacccac tcggggtctc agtgtcctca gttgtaacat tagagagatg 2090

gcatcaatgc ttgataagga cccttctata attccaattg ccagttatcc 2140

aaactctgat tcggtggtcg agctggcctc gtgttcttat ctgctaaccc 2190

tgtcttacct tccagcctca gttaagtcaa atcaagggct atgtcattgc 2240

tgaatgtcat ggggggcaac tgcttgccct ccaccctata gtatctattt 2290

tatgaaattc caagaaggga tgaataaata aatctcttgg atgctgcgtc 2340

tggcagtctt cacgggtggt tttcaaagca gaaaaaaaaa aaaaaaaaaa 2390

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a 2431

<210> 11
<211> 768
<212> PRT
<213> Homo sapiens

<400> 11
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Arg Asp Ala Glu Arg Arg Tyr Thr Ser Ser Ser Ala Asp Ser Glu
20 25 30

Glu Gly Lys Ala Pro Gln Lys Ser Tyr Ser Ser Ser Glu Thr Leu
35 40 45

```
Lys Ala Tyr Asp Gln Asp Ala Arg Leu Ala Tyr Gly Ser Arg Val
                50                  55                  60

Lys Asp Ile Val Pro Gln Glu Ala Glu Glu Phe Cys Arg Thr Gly
                65                  70                  75

Ala Asn Phe Thr Leu Arg Glu Leu Gly Leu Glu Glu Val Thr Pro
                80                  85                  90

Pro His Gly Thr Leu Tyr Arg Thr Asp Ile Gly Leu Pro His Cys
                95                 100                 105

Gly Tyr Ser Met Gly Ala Gly Ser Asp Ala Asp Met Glu Ala Asp
               110                 115                 120

Thr Val Leu Ser Pro Glu His Pro Val Arg Leu Trp Gly Arg Ser
               125                 130                 135

Thr Arg Ser Gly Arg Ser Ser Cys Leu Ser Ser Arg Ala Asn Ser
               140                 145                 150

Asn Leu Thr Leu Thr Asp Thr Glu His Glu Asn Thr Glu Thr Asp
               155                 160                 165

His Pro Gly Gly Leu Gln Asn His Ala Arg Leu Arg Thr Pro Pro
               170                 175                 180

Pro Pro Leu Ser His Ala His Thr Pro Asn Gln His His Ala Ala
               185                 190                 195

Ser Ile Asn Ser Leu Asn Arg Gly Asn Phe Thr Pro Arg Ser Asn
               200                 205                 210

Pro Ser Pro Ala Pro Thr Asp His Ser Leu Ser Gly Glu Pro Pro
               215                 220                 225

Ala Gly Gly Ala Gln Glu Pro Ala His Ala Gln Glu Asn Trp Leu
               230                 235                 240

Leu Asn Ser Asn Ile Pro Leu Glu Thr Arg Asn Leu Gly Lys Gln
               245                 250                 255

Pro Phe Leu Gly Thr Leu Gln Asp Asn Leu Ile Glu Met Asp Ile
               260                 265                 270

Leu Gly Ala Ser Arg His Asp Gly Ala Tyr Ser Asp Gly His Phe
               275                 280                 285

Leu Phe Lys Pro Gly Gly Thr Ser Pro Leu Phe Cys Thr Thr Ser
               290                 295                 300

Pro Gly Tyr Pro Leu Thr Ser Ser Thr Val Tyr Ser Pro Pro Pro
               305                 310                 315

Arg Pro Leu Pro Arg Ser Thr Phe Ala Arg Pro Ala Phe Asn Leu
               320                 325                 330
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Lys Lys Pro Ser Lys Tyr Cys Asn Trp Lys Cys Ala Ala Leu Ser
335                 340                 345

Ala Ile Val Ile Ser Ala Thr Leu Val Ile Leu Leu Ala Tyr Phe
350                 355                 360

Val Ala Met His Leu Phe Gly Leu Asn Trp His Leu Gln Pro Met
365                 370                 375

Glu Gly Gln Met Tyr Glu Ile Thr Glu Asp Thr Ala Ser Ser Trp
380                 385                 390

Pro Val Pro Thr Asp Val Ser Leu Tyr Pro Ser Gly Gly Thr Gly
395                 400                 405

Leu Glu Thr Pro Asp Arg Lys Gly Lys Gly Thr Thr Glu Gly Lys
410                 415                 420

Pro Ser Ser Phe Phe Pro Glu Asp Ser Phe Ile Asp Ser Gly Glu
425                 430                 435

Ile Asp Val Gly Arg Arg Ala Ser Gln Lys Ile Pro Pro Gly Thr
440                 445                 450

Phe Trp Arg Ser Gln Val Phe Ile Asp His Pro Val His Leu Lys
455                 460                 465

Phe Asn Val Ser Leu Gly Lys Ala Ala Leu Val Gly Ile Tyr Gly
470                 475                 480

Arg Lys Gly Leu Pro Pro Ser His Thr Gln Phe Asp Phe Val Glu
485                 490                 495

Leu Leu Asp Gly Arg Arg Leu Leu Thr Gln Glu Ala Arg Ser Leu
500                 505                 510

Glu Gly Thr Pro Arg Gln Ser Arg Gly Thr Val Pro Pro Ser Ser
515                 520                 525

His Glu Thr Gly Phe Ile Gln Tyr Leu Asp Ser Gly Ile Trp His
530                 535                 540

Leu Ala Phe Tyr Asn Asp Gly Lys Glu Ser Glu Val Val Ser Phe
545                 550                 555

Leu Thr Thr Ala Ile Ala Leu Pro Pro Arg Leu Lys Glu Met Lys
560                 565                 570

Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu
575                 580                 585

Thr Ser Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn
590                 595                 600

Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile
605                 610                 615
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Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser
620 625 630

Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
635 640 645

Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn
650 655 660

Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val
665 670 675

Ser Ser Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala
680 685 690

Asn Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His
695 700 705

Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly
710 715 720

Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
725 730 735

Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn
740 745 750

Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro Phe Leu Ser
755 760 765

Leu Pro Glu
768

<210> 12
<211> 3111
<212> DNA
<213> Homo sapiens

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gggtcgtggg cacctccaga aaagatcccg caccatcctc caggatccaa 100

tggccttgga gagagggctg cagggcccac ggacattgct gactcttcag 150

aacgtgctga catggagcca ggtagactga aattatcatg tgtccaaatt 200

aaaattgcat acttcaagga ttatttgaag gactattctt agacccttt 250

aagaagattt aaagaaaaac cactcggccc tgagtgcggc gaggaccctg 300

tttgtggatg tggaggagcg cgggccggag gcc atg gac gtg 342
Met Asp Val
1

aag gag agg aag cct tac cgc tcg ctg acc cgg cgc cgc 381

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Lys Glu Arg Lys Pro Tyr Arg Ser Leu Thr Arg Arg Arg
      5                  10                  15

gac gcc gag cgc cgc tac acc agc tcg tcc gcg gac agc 420
Asp Ala Glu Arg Arg Tyr Thr Ser Ser Ser Ala Asp Ser
5               20                  25

gag gag ggc aaa gcc ccg cag aaa tcg tac agc tcc agc 459
Glu Glu Gly Lys Ala Pro Gln Lys Ser Tyr Ser Ser Ser
 30                  35                  40

gag acc ctg aag gcc tac gac cag gac gcc cgc cta gcc 498
Glu Thr Leu Lys Ala Tyr Asp Gln Asp Ala Arg Leu Ala
          45                  50                  55

tat ggc agc cgc gtc aag gac att gtg ccg cag gag gcc 537
Tyr Gly Ser Arg Val Lys Asp Ile Val Pro Gln Glu Ala
                  60                  65

gag gaa ttc tgc cgc aca ggt gcc aac ttc acc ctg cgg 576
Glu Glu Phe Cys Arg Thr Gly Ala Asn Phe Thr Leu Arg
     70                  75                  80

gag ctg ggg ctg gaa gaa gta acg ccc cct cac ggg acc 615
Glu Leu Gly Leu Glu Glu Val Thr Pro Pro His Gly Thr
               85                  90

ctg tac cgg aca gac att ggc ctc ccc cac tgc ggc tac 654
Leu Tyr Arg Thr Asp Ile Gly Leu Pro His Cys Gly Tyr
 95                  100                 105

tcc atg ggg gct ggc tct gat gcc gac atg gag gct gac 693
Ser Met Gly Ala Gly Ser Asp Ala Asp Met Glu Ala Asp
        110                 115                 120

acg gtg ctg tcc cct gag cac ccc gtg cgt ctg tgg ggc 732
Thr Val Leu Ser Pro Glu His Pro Val Arg Leu Trp Gly
                125                 130

cgg agc aca cgg tca ggg cgc agc tcc tgc ctg tcc agc 771
Arg Ser Thr Arg Ser Gly Arg Ser Ser Cys Leu Ser Ser
    135                 140                 145

cgg gcc aat tcc aat ctc aca ctc acc gac acc gag cat 810
Arg Ala Asn Ser Asn Leu Thr Leu Thr Asp Thr Glu His
            150                 155

gaa aac act gag act gat cat ccg ggc ggc ctg cag aac 849
Glu Asn Thr Glu Thr Asp His Pro Gly Gly Leu Gln Asn
160                 165                 170

cac gcg cgg ctc cgg acg ccg ccg ccg ccg ctc tcg cac 888
His Ala Arg Leu Arg Thr Pro Pro Pro Pro Leu Ser His
        175                 180                 185

gcc cac acc ccc aac cag cac cac gcg gcc tcc att aac 927
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```
Ala His Thr Pro Asn Gln His His Ala Ala Ser Ile Asn
                  190                 195

tcc ctg aac cgg ggc aac ttc acg ccg agg agc aac ccc 966
Ser Leu Asn Arg Gly Asn Phe Thr Pro Arg Ser Asn Pro
    200                 205                 210

agc ccg gcc ccc acg gac cac tcg ctc tcc gga gag ccc 1005
Ser Pro Ala Pro Thr Asp His Ser Leu Ser Gly Glu Pro
            215                 220

cct gcc ggc ggc gcc cag gag cct gcc cac gcc cag gag 1044
Pro Ala Gly Gly Ala Gln Glu Pro Ala His Ala Gln Glu
225                 230                 235

aac tgg ctg ctc aac agc aac atc ccc ctg gag acc aga 1083
Asn Trp Leu Leu Asn Ser Asn Ile Pro Leu Glu Thr Arg
        240                 245                 250

aac cta ggc aag cag cca ttc cta ggg aca ttg cag gac 1122
Asn Leu Gly Lys Gln Pro Phe Leu Gly Thr Leu Gln Asp
                  255                 260

aac ctc att gag atg gac att ctc ggc gcc tcc cgc cat 1161
Asn Leu Ile Glu Met Asp Ile Leu Gly Ala Ser Arg His
    265                 270                 275

gat ggg gct tac agt gac ggg cac ttc ctc ttc aag cct 1200
Asp Gly Ala Tyr Ser Asp Gly His Phe Leu Phe Lys Pro
            280                 285

gga ggc acc tcc ccg ctc ttc tgc acc aca tca cca ggg 1239
Gly Gly Thr Ser Pro Leu Phe Cys Thr Thr Ser Pro Gly
290                 295                 300

tac cca ctg acg tcc agc aca gtg tac tct cct ccg ccc 1278
Tyr Pro Leu Thr Ser Ser Thr Val Tyr Ser Pro Pro Pro
        305                 310                 315

cga ccc ctg ccc cgc agc acc ttc gcc cgg ccg gcc ttt 1317
Arg Pro Leu Pro Arg Ser Thr Phe Ala Arg Pro Ala Phe
                  320                 325

aac ctc aag aag ccc tcc aag tac tgt aac tgg aag tgc 1356
Asn Leu Lys Lys Pro Ser Lys Tyr Cys Asn Trp Lys Cys
    330                 335                 340

gca gcc ctg agc gcc atc gtc atc tca gcc act ctg gtc 1395
Ala Ala Leu Ser Ala Ile Val Ile Ser Ala Thr Leu Val
            345                 350

atc ctg ctg gca tac ttt gtg gcc atg cac ctg ttt ggc 1434
Ile Leu Leu Ala Tyr Phe Val Ala Met His Leu Phe Gly
355                 360                 365

cta aac tgg cac ctg cag ccg atg gag ggg cag atg tat 1473
```

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Leu Asn Trp His Leu Gln Pro Met Glu Gly Gln Met Tyr
        370                 375                 380

gag atc acg gag gac aca gcc agc agt tgg cct gtg cca 1512
Glu Ile Thr Glu Asp Thr Ala Ser Ser Trp Pro Val Pro
                385                 390

acc gac gtc tcc cta tac ccc tca ggg ggc act ggc tta 1551
Thr Asp Val Ser Leu Tyr Pro Ser Gly Gly Thr Gly Leu
    395                 400                 405

gag acc cct gac agg aaa ggc aaa gga acc aca gaa gga 1590
Glu Thr Pro Asp Arg Lys Gly Lys Gly Thr Thr Glu Gly
            410                 415

aag ccc agt agt ttc ttt cca gag gac agt ttc ata gat 1629
Lys Pro Ser Ser Phe Phe Pro Glu Asp Ser Phe Ile Asp
420                 425                 430

tct gga gaa att gat gtg gga agg cga gct tcc cag aag 1668
Ser Gly Glu Ile Asp Val Gly Arg Arg Ala Ser Gln Lys
        435                 440                 445

att cct cct ggc act ttc tgg aga tct caa gtg ttc ata 1707
Ile Pro Pro Gly Thr Phe Trp Arg Ser Gln Val Phe Ile
                450                 455

gac cat cct gtg cat ctg aaa ttc aat gtg tct ctg gga 1746
Asp His Pro Val His Leu Lys Phe Asn Val Ser Leu Gly
    460                 465                 470

aag gca gcc ctg gtt ggc att tat ggc aga aaa ggc ctc 1785
Lys Ala Ala Leu Val Gly Ile Tyr Gly Arg Lys Gly Leu
            475                 480

cct cct tca cat aca cag ttt gac ttt gtg gag ctg ctg 1824
Pro Pro Ser His Thr Gln Phe Asp Phe Val Glu Leu Leu
485                 490                 495

gat ggc agg agg ctc cta acc cag gag gcg cgg agc cta 1863
Asp Gly Arg Arg Leu Leu Thr Gln Glu Ala Arg Ser Leu
        500                 505                 510

gag ggg acc ccg cgc cag tct cgg gga act gtg ccc ccc 1902
Glu Gly Thr Pro Arg Gln Ser Arg Gly Thr Val Pro Pro
                515                 520

tcc agc cat gag aca ggc ttc atc cag tat ttg gat tca 1941
Ser Ser His Glu Thr Gly Phe Ile Gln Tyr Leu Asp Ser
    525                 530                 535

gga atc tgg cac ttg gct ttt tac aat gac gga aag gag 1980
Gly Ile Trp His Leu Ala Phe Tyr Asn Asp Gly Lys Glu
            540                 545

tca gaa gtg gtt tcc ttt ctc acc act gcc att gcc ttg 2019
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Ser Glu Val Val Ser Phe Leu Thr Thr Ala Ile Ala Leu
550                 555                 560

cct ccc cga ttg aaa gag atg aaa agc cag gaa tcg gct 2058
Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu Ser Ala
     565                 570                 575

gca ggt tcc aaa cta gtc ctt cgg tgt gaa acc agt tct 2097
Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser
                580                 585

gaa tac tcc tct ctc aga ttc aag tgg ttc aag aat ggg 2136
Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly
    590                 595                 600

aat gaa ttg aat cga aaa aac aaa cca caa aat atc aag 2175
Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys
            605                 610

ata caa aaa aag cca ggg aag tca gaa ctt cgc att aac 2214
Ile Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn
615                 620                 625

aaa gca tca ctg gct gat tct gga gag tat atg tgc aaa 2253
Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys
     630                 635                 640

gtg atc agc aaa tta gga aat gac agt gcc tct gcc aat 2292
Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn
                645                 650

atc acc atc gtg gaa tca aac gag atc atc act ggt atg 2331
Ile Thr Ile Val Glu Ser Asn Glu Ile Ile Thr Gly Met
    655                 660                 665

cca gcc tca act gaa gga gca tat gtg tct tca gag tct 2370
Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu Ser
            670                 675

ccc att aga ata tca gta tcc aca gaa gga gca aat act 2409
Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
680                 685                 690

tct tca tct aca tct aca tcc acc act ggg aca agc cat 2448
Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His
     695                 700                 705

ctt gta aaa tgt gcg gag aag gag aaa act ttc tgt gtg 2487
Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val
                710                 715

aat gga ggg gag tgc ttc atg gtg aaa gac ctt tca aac 2526
Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn
    720                 725                 730

ccc tcg aga tac ttg tgc aag tgc cca aat gag ttt act 2565
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Pro Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr
735 740

ggt gat cgc tgc caa aac tac gta atg gcc agc ttc tac 2604
Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr
745 750 755

agt acg tcc act ccc ttt ctg tct ctg cct gaa tag 2640
Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro Glu
760 765 768

gagcatgctc agttggtgct gctttcttgt tgctgcatct cccctcagat 2690

tccacctaga gctagatgtg tcttaccaga tctaatattg actgcctctg 2740

cctgtcgcat gagaacatta acaaaagcaa ttgtattact tcctctgttc 2790

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cagtgaaata tgataataaa ggcatttcaa agtctcactt ttattgataa 2940

aataaaaatc attctactga acagtccatc ttctttatac aatgaccaca 2990

tcctgaaaag ggtgttgcta agctgtaacc gatatgcact tgaaatgatg 3040

gtaagttaat tttgattcag aatgtgttat ttgtcacaaa taaacataat 3090

aaaaggaaaa aaaaaaaaaa a 3111

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tcttcatctg agcagacacc agcttcagat gctcgaggtg agaaacatgc 150

ctttcagttt gggctactgg tttacttaat taatcagccg gcagctccgt 200

cgatctattt tcgtccctgt cctcttgacg agcccgggat ggtttggagt 250

agcatttaaa agaactagaa aagtggccca gaaacagcag cttaaagaat 300

tattacgata tactttgatt ttgtagttgc taggagcttt tcttcccccc 350

ttgcatcttt ctgaactctt cttgatttta ataatggcct tggacttgga 400

cgatttatcg atttcccccc gtaagatgct gtatcatttg gttgggggggg 450

cctctgcgtg gtaatggacc gtgagagcgg ccaggccttc ttctggaggt 500

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gagccg  atg gag att tat tcc cca gac atg tct gag gtc 539
        Met Glu Ile Tyr Ser Pro Asp Met Ser Glu Val
          1               5                  10

gcc gcc gag agg tcc tcc agc ccc tcc act cag ctg agt 578
Ala Ala Glu Arg Ser Ser Ser Pro Ser Thr Gln Leu Ser
              15                  20

gca gac cca tct ctt gat ggg ctt ccg gca gca gaa gac 617
Ala Asp Pro Ser Leu Asp Gly Leu Pro Ala Ala Glu Asp
 25                  30                  35

atg cca gag ccc cag act gaa gat ggg aga acc cct gga 656
Met Pro Glu Pro Gln Thr Glu Asp Gly Arg Thr Pro Gly
          40                  45                  50

ctc gtg ggc ctg gcc gtg ccc tgc tgt gcg tgc cta gaa 695
Leu Val Gly Leu Ala Val Pro Cys Cys Ala Cys Leu Glu
                  55                  60

gct gag cgc ctg aga ggt tgc ctc aac tca gag aaa atc 734
Ala Glu Arg Leu Arg Gly Cys Leu Asn Ser Glu Lys Ile
     65                  70                  75

tgc att gtc ccc atc ctg gct tgc ctg gtc agc ctc tgc 773
Cys Ile Val Pro Ile Leu Ala Cys Leu Val Ser Leu Cys
              80                  85

ctc tgc atc gcc ggc ctc aag tgg gta ttt gtg gac aag 812
Leu Cys Ile Ala Gly Leu Lys Trp Val Phe Val Asp Lys
 90                  95                  100

atc ttt gaa tat gac tct cct act cac ctt gac cct ggg 851
Ile Phe Glu Tyr Asp Ser Pro Thr His Leu Asp Pro Gly
         105                 110                 115

ggg tta ggc cag gac cct att att tct ctg gac gca act 890
Gly Leu Gly Gln Asp Pro Ile Ile Ser Leu Asp Ala Thr
                 120                 125

gct gcc tca gct gtg tgg gtg tcg tct gag gca tac act 929
Ala Ala Ser Ala Val Trp Val Ser Ser Glu Ala Tyr Thr
     130                 135                 140

tca cct gtc tct agg gct caa tct gaa agt gag gtt caa 968
Ser Pro Val Ser Arg Ala Gln Ser Glu Ser Glu Val Gln
              145                 150

gtt aca gtg caa ggt gac aag gct gtt gtc tcc ttt gaa 1007
Val Thr Val Gln Gly Asp Lys Ala Val Val Ser Phe Glu
155                 160                 165

cca tca gcg gca ccg aca ccg aag aat cgt att ttt gcc 1046
Pro Ser Ala Ala Pro Thr Pro Lys Asn Arg Ile Phe Ala
         170                 175                 180
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ttt tct ttc ttg ccg tcc act gcg cca tcc ttc cct tca 1085
Phe Ser Phe Leu Pro Ser Thr Ala Pro Ser Phe Pro Ser
                185                 190

ccc acc cgg aac cct gag gtg aga acg ccc aag tca gca 1124
Pro Thr Arg Asn Pro Glu Val Arg Thr Pro Lys Ser Ala
    195                 200                 205

act cag cca caa aca aca gaa act aat ctc caa act gct 1163
Thr Gln Pro Gln Thr Thr Glu Thr Asn Leu Gln Thr Ala
            210                 215

cct aaa ctt tct aca tct aca tcc acc act ggg aca agc 1202
Pro Lys Leu Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser
220                 225                 230

cat ctt gta aaa tgt gcg gag aag gag aaa act ttc tgt 1241
His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys
        235                 240                 245

gtg aat gga ggg gag tgc ttc atg gtg aaa gac ctt tca 1280
Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser
                250                 255

aac ccc tcg aga tac ttg tgc aag tgc cca aat gag ttt 1319
Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe
    260                 265                 270

act ggt gat cgc tgc caa aac tac gta atg gcc agc ttc 1358
Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe
            275                 280

tac agt acg tcc act ccc ttt ctg tct ctg cct gaa taggag 1400
Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro Glu
285                 290                 295 296

catgctcagt tggtgctgct ttcttgttgc tgcatctccc ctcagattcc 1450

acctagagct agatgtgtct taccagatct aatattgact gcctctgcct 1500

gtcgcatgag aacattaaca aaagcaattg tattacttcc tctgttcgcg 1550

actagttggc tctgagatac taataggtgt gtgaggctcc ggatgtttct 1600

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Ser Ser Ser Pro Ser Thr Gln Leu Ser Ala Asp Pro Ser Leu Asp
            20                  25                  30

Gly Leu Pro Ala Ala Glu Asp Met Pro Glu Pro Gln Thr Glu Asp
        35                  40                  45

Gly Arg Thr Pro Gly Leu Val Gly Leu Ala Val Pro Cys Cys Ala
    50                  55                  60

Cys Leu Glu Ala Glu Arg Leu Arg Gly Cys Leu Asn Ser Glu Lys
65                  70                  75

Ile Cys Ile Val Pro Ile Leu Ala Cys Leu Val Ser Leu Cys Leu
            80                  85                  90

Cys Ile Ala Gly Leu Lys Trp Val Phe Val Asp Lys Ile Phe Glu
        95                  100                 105

Tyr Asp Ser Pro Thr His Leu Asp Pro Gly Gly Leu Gly Gln Asp
    110                 115                 120

Pro Ile Ile Ser Leu Asp Ala Thr Ala Ala Ser Ala Val Trp Val
125                 130                 135

Ser Ser Glu Ala Tyr Thr Ser Pro Val Ser Arg Ala Gln Ser Glu
140                 145                 150

Ser Glu Val Gln Val Thr Val Gln Gly Asp Lys Ala Val Val Ser
155                 160                 165

Phe Glu Pro Ser Ala Ala Pro Thr Pro Lys Asn Arg Ile Phe Ala
170                 175                 180

Phe Ser Phe Leu Pro Ser Thr Ala Pro Ser Phe Pro Ser Pro Thr
185                 190                 195

Arg Asn Pro Glu Val Arg Thr Pro Lys Ser Ala Thr Gln Pro Gln
200                 205                 210

Thr Thr Glu Thr Asn Leu Gln Thr Ala Pro Lys Leu Ser Thr Ser
215                 220                 225

Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala Glu Lys
230                 235                 240

Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys Phe Met Val Lys
245                 250                 255
```

```
Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu
                260                 265                 270

Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr
                275                 280                 285

Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro Glu
                290                 295 296
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